WESTERN-EUROPEAN INFLUENCES ON THE POST-BYZANTINE ICON PAINTING TECHNIQUE OF CRETE & THE ISLANDS OF IONION

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Ithaca...

When you set out on your journey to Ithaca,
pray that the road is long,
full of adventure, full of knowledge.
The Lestrygonians and the Cyclops,
the angry Poseidon -- do not fear them:

You will never find such as these on your path,
if your thoughts remain lofty, if a fine
emotion touches your spirit and your body.
The Lestrygonians and the Cyclops,
the fierce Poseidon you will never encounter,
if you do not carry them within your soul,
if your soul does not set them up before you.

Pray that the road is long.
That the summer mornings are many, when,
with such pleasure, with such joy
you will enter ports seen for the first time;
stop at Phoenician markets,
and purchase fine merchandise,
mother-of-pearl and coral, amber and ebony,
and sensual perfumes of all kinds,
as many sensual perfumes as you can;
visit many Egyptian cities,
to learn and learn from scholars.

Always keep Ithaca in your mind.
To arrive there is your ultimate goal.
But do not hurry the voyage at all.
It is better to let it last for many years;
and to anchor at the island when you are old,
rich with all you have gained on the way,
not expecting that Ithaca will offer you riches.

Ithaca has given you the beautiful voyage.
Without her you would have never set out on the road.
She has nothing more to give you.

And if you find her poor, Ithaca has not deceived you.
Wise as you have become, with so much experience,
you must already have understood what Ithacas mean.

C. P. Cavafy 1911

ABSTRACT

ELENI KOULOUMPI **2007**
WESTERN-EUROPEAN INFLUENCES ON THE POST-BYZANTINE ICON PAINTING TECHNIQUE OF CRETE & THE ISLANDS OF IONION

The post-Byzantine Art was not only one of the most important artistic movements of Greece, but it was also a period in which serious and decisive changes took place. The most important change was the change of the materials and techniques; from egg yolk to drying oil and from panel paintings on wood to easel paintings on canvas.

A series of a hundred and twenty one panel paintings, representative samples of this period (late 15th century up to the early 19th century) and from the work of the most important artists, who represent this period but who also contributed to the evolution of the later pictorial art were studied in the current research. Trying to find evidence of time and location for changes in practice between Constantinople, Greece and Venice, two hundred and one samples were collected from Crete, Athens Thessaloniki, Cephalonia, Zakynthos and Patmos. The aim was to provide time/location data slices concerning the creation of the artwork and to identify the materials present in order to be able to detect any possible changes to the technique. The research proved to be quite a complicated task, not only due to the nature of the materials studied, but also due to the limited samples and sample quantity available.

A multi-method approach was employed in order to characterise the artists' materials. The analytical means with which the investigation was carried out were: Energy Dispersive X-ray Analysis, Fourier Transform Infrared Microscopy and Gas Chromatography. Additionally, other two techniques were used to offer complementary information wherever necessary: Raman microscopy and staining of cross-sections.

Pure binding media, as well as emulsions were detected, while the nature of the selected pigments was identified. The artistic changes that took place in the post-Byzantine of painting did record on the techniques available. It is obvious that while in the Byzantine art the main binder is egg yolk, immediately after the formation of the post-Byzantine School egg/oil emulsion and drying oil are introduced. In the 16th century there seems to be a rise in the use of drying oils, either in the form of an additive layer over a proteinaceous one or in the form of a single layer binder. The 17th century establishes the use of emulsions, until the 18th century where the use of drying oils prevails. This unexpected sequence of changes was repeated through all the schools studied, it seems reproducible through each series of samples and is not being influenced by other considerations.

The results of this research led to one main conclusion: Western Europe did affect the icon painters. It gave them the examples and the materials for them to free themselves and move on to more contemporary styles.

Only a fraction of the data obtained has been published. The results of this study may have thrown some light onto this dark transitional period of the Hellenic art, but as research never ends, new questions have been born, for scientists to answer.

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ABBREVIATIONS

AA	Amino acids
ECF	Ethyl chloroformate
FA	Fatty acid
FTIR	Fourrier Transform Infrared spectroscopy
GC	Gas chromatography
GC-FID	Gas chromatography – Flame ionisation detector
GC-MS	Gas chromatography-mass spectroscopy
ICOM	International Committee of Museums
UKIC	United Kingdom Institute for Conservation
A	Alanine
G	Glycine
V	Valine
L	Leucine
I	Isoleucine
P	Proline
OH-P	Hydroxyproline
C9	Azelaic acid
C16	Palmitic acid
C18:1	Oleic acid
C18	Stearic acid

ACTIVITIES AND TRAINING UNDERTAKEN DURING THE RESEARCH PROJECT

List of publications

1. Kouloumpi E., Vandenabeele P., Lawson G., Pavlidis V. & Moens L. (2007) Analysis of post-Byzantine icons from the Church of the Assumption in Cephalonia, Ionian Islands, Greece: A multi-method approach. *Analytica Chimica Acta*, 598, pp.169 –179.

2. Kouloumpi E., Lawson G. & Pavlidis V., (2007) The Contribution of Gas Chromatography to the Resynthesis of the Post-Byzantine Artist's Technique, *Analytical and Bioanalytical Chemistry*, 387, 803 – 812.

3. Eleni Kouloumpi, Konstantinos Stoupathis, Sotiriou Ioannis, Grigorios Selianitis, Graham Lawson (2006) The Coronation of the Virgin : A new acquisition of the Holy monastery of St Theologos of Patmos : Physicochemical Research & Conservation Treatment of the Artefact. *In proceedings of the Icons: Approaches to Research, Conservation and Ethical Issues*, Athens, 3-7 December 2006, New Benaki Museum.

4. Moutsatsou A., Kouloumpi E., Terlixi A., Doulgeridis M. (2006) Physicochemical Study of Icons at the National Gallery of Athens: A Routine Process. *In the proceeding of the Icon and Portrait International Conference*, Cairo, September, 2006, pp.116-125.

5. Kouloumpi E., Vandenabeele P., Lawson G., Pavlidis V., (2006) Physicochemical Study of the Western European Influences on Post-Byzantine Panel Painting Technique (poster). *In 36th International Symposium on Archaeometry, ISA 2006,* Quebec City, May 2006, Canada. [awarded as the best poster voted by the attendees].

6. Kouloumpi E., Lawson G., Pavlidis V., (2006) Western European Influences on Post-Byzantine panel painting technique through binding media identification. In Mendoza D., Arenas J., A., Ruvalcaba J., L., Rodriguez V., *La Ciencia de Materiales y su Impacto en la Arquelogia, Volumen III,* XIV International Materials Research Congress, 21-25 August 2005, Mexico, Innovacion Editorial Lagares de Mexico pp.105-122.

7. Ioakimoglou, E., Kavalieratou, E., Zevgiti, S., Stoupathis, K., & Kouloumpi, E. (2003) Identification of proteinaceous binding media from post-byzantine mural paintings with chromatographic techniques. In: *Postprints of 4th Symposium of the Hellenic Society for Archaeometry, 28 – 31 May,* Athens.

Additional training

1. "COST Action G8, Non-destructive testing and analysis of museum objects", training course, held at Intercollege, Nicosia, Cyprus, 18-20 May 2006.

2. "Intellectual property right and ethics", research training course held at De Montfort University, Leicester, 14th March 2006.

3. "Writing skills", training course, research training course held at De Montfort University, Leicester, 13th March 2006.

4. "Presenting your research to an audience", research training course held at De Montfort University, Leicester, 13th March 2006.

5. "Finishing your thesis and preparing for the Viva", research training course held at De Montfort University, Leicester, 9th March 2006.

6. "Lasers for the preservation of cultural heritage", training course, held at the Foundation of Research and Technology – Hellas (Forth), Heraklion, Greece, 7 – 11 February 2005.

Lectures given

1. "A forensic examination of post-Byzantine icons" lecture held in De Montfort University, 3rd February 2005.

2. "Conservation Science" lecture held in London Metropolitan University, 31st January 2005.

3. "Conservation and Restoration of panel paintings" tutorial and practice, held in Petra institute of vocational training, academic years 2001-2004, 288 hours per annum.

4. "Environmental Monitoring and control of painted artefacts" tutorial, held in Petra institute of vocational training, academic years 2003-2004, 50 hours per annum.

5. "Conservation and Restoration of panel paintings" tutorial, held in Peristeri institute of vocational training, academic years 2001-2002, 96 hours.

Participation in research programs

1. European Commission funded research program "Multifunctional Encoding System for Assessment of Movable Cultural Heritage", (2005 – 2008).
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2. European Commission funded research program "Laser for Stonework Restoration" (2003 – 2006).
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Conferences attended

3. Conference with subject "Icons: Approaches to Research, Conservation and Ethical Issues". Athens, 3-7 December 2006, New Benaki Museum, Greece.

4. Conference with subject "La Ciencia de Materiales y su Impacto en la Arquelogia", XIV International Materials Research Congress. 21-25 August 2005, Cancun, Mexico.

5. Conference with subject "ICOM, Recent Preoccupations Concerning Textiles, Leather, Legislation". 21-24 April 2004, Byzantine & Christian Museum of Athens, Greece.

6. Conference with subject "4th Symposium of Archaeometry". Hellenic Society of Archaeometry – National Institute of Research, 28-31 May 2003, Greece.

7. Conference with subject "Conservation and Exhibition of Restored Artefacts: Technical Problems – Aesthetic Problems". Byzantine & Christian Museum of Athens, 29th of January 2003, Greece.

8. Symposium with subject "Study on the Organic Materials of Archaeology and Works of Art" Hellenic Society of Archaeometry – National Institute of Research, 15 January 2002, Greece.

9. Symposium with subject "Byzantium as an Ecumenical Society", Section of Byzantine and Post-Byzantine Monuments-Ministry of Culture / Institute of Byzantine Research – National Institute of Research, 29 November – 2 December 2001, Greece.

AUTHOR'S DECLARATION

The work discussed in this thesis was carried out whilst the author was registered with the Faculty of Health & Life Sciences, De Montfort University, Leicester, between December 2001 and September 2007. It is the original work of the author.

This thesis has not been submitted for any other degree at this or any other university.

Eleni Kouloumpi

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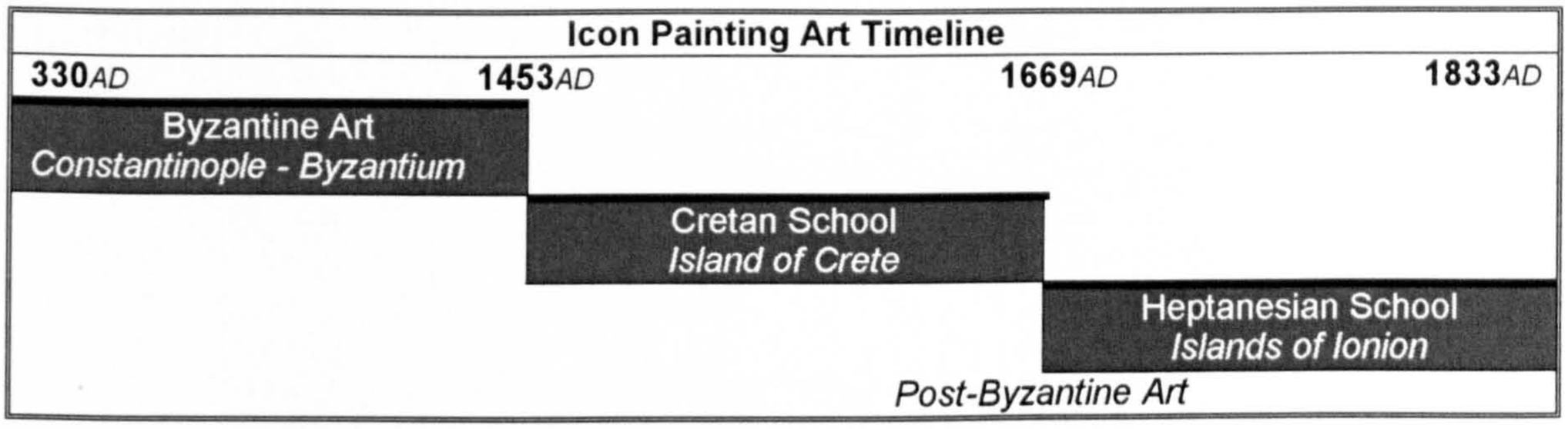
CHAPTER 1
INTRODUCTION

1.1 Background and context

According to historians (Hatzidakis, 1987; Kritsotakis, 1934), the transfer of the capital of the Roman Empire from Rome to Constantinople by Constantine the Great in 330 AD was the beginning of the Byzantine Empire, which lasted from the 4th to the 15th century (1453) when the Ottomans invaded Constantinople. During these centuries a Christian art very strictly ruled, in terms of pictorial characteristics was developed, the famous Byzantine Art. 1453 saw the end of the Byzantine period and the beginning of the post-Byzantine era, which lasted until nearly 1830, which is taken as the end of the post Byzantine period.

Figure 1.1 Icon painting timeline



During the post-Byzantine era, a number of schools were developed (Figure 1.1) with the most important being the Cretan School (from the island of Crete) (Kritsotakis, 1934). This School flourished whilst Crete was under Venetian Rule. Initially, the Cretan artists were following the strict rules of the Byzantine art of iconography, but through cultural exchanges with Venice, they gradually adopted western characteristics.

In 1669, Crete was surrendered to the Ottomans and the glorious days of the Cretan School came to an end. In following years Cretan artists migrated to several areas of Greece, mainly the islands of Ionion (Corfu, Cephalonia and

Zakynthos) and to Venice. Again they were following the Byzantine rules but with western characteristics.

Gradually, the cultural centre on the islands of Ionion (Heptanesian School) came to replace the Cretan School but it never equalled its glory. However, during that period Panayotis Doxaras (1699-1732) made his appearance in the Ionion. He was very deeply influenced by the Italian art and all his work was based on those influences. With the death of the Cretan School followers and the influence of Doxaras, the western Italian mentality or practices predominated the Byzantine art of the second quarter of the 18th century (Demus, 1970; Dizikirikis, 1996).

This post-Byzantine era (Figure 1.2) was the transitional period of Greek pictorial art, in which the artists (Appendix I) passed from the egg tempera to oil paintings. Of course, the art of icon painting never "died", but its technical evolution ceased.

Figure 1.2 The route of the Post-Byzantine art. The dash-lines indicate the migration of the artists and the movement of the paint style, while the dotted lines the journeys to and interactions with Venice.

1.2 The analyses of materials of cultural property

Scientific analyses of works of art not only becomes more and more important, but also a necessity in the field of art. Conservation science is a relatively new discipline, which occurs from the need to understand the works of art. Our cultural property is part of human history and civilisation and in order to be understood, it has to be studied from all points of view.

Analysis of an object can provide information on the chemical structure of the materials present, methods of construction and manufacture, dating and provenance. Moreover, it can provide an understanding of the degradation processes of the materials, hence the condition of the object analysed.

Until very recently, the job of a conservator was based simply on practical skills. There was no understanding whatsoever on the science behind an artefact and its treatment was based on recipes. People knew that shellac, for example, can be removed from an object with alcohol, but they did not know why. And if the varnish was not shellac, then they would try all sorts of solvents, until they found the one that would affect the layer. Today, a conservator can easily find out the nature of a varnish and consequently, the solvent to be used. Similar information can be obtained that will lead to a choice of a suitable method of treatment. Furthermore, the materials that will be used for a treatment will be of known composition, with known properties and with known effect on the artefact. Finally, the understanding of an artefact can lead to suitable display and storage conditions.

Conservation science and scientific analyses of works of art, generally, provide information that can be used and is being used by a variety of scientists. Art historians, archaeologists, conservators and analytical chemists can use this information, in order to gain a better understanding of the cultural continuity and evolution.

Antique dealers can use analyses for dating and authentication. Original artefacts have been coated in the past with a number of layers, in order to produce a freshly made appearance that would allow easy export. How would this artefact be authenticated, if science was not used? The artefact could become the subject of illegal art dealing.

The examples mentioned above highlight the importance of science in art. It is however very important to remember that even an isolated analysis adds something to human knowledge.

1.2.1 Ethical Considerations

However, before taking a sample for analysis, the objectives should always be defined. What do we need to find out? Which method is required? What sample is suitable for that method? How much sample is needed? Can the sample be re-used? Pigment identification, for example, requires a relatively pure sample taken from the paint layer, while the examination of a layer structure requires a section taken from the artefact.

Researchers need information, but what about the sample taken? Conservation aims to preserve our cultural property and its integrity. Every time conservation scientists and analytical chemists plan to do an analysis, they should bear in mind that sampling affects the integrity of an artefact. They should also act according to codes of ethics as set by many organisations like the UKIC (1996). Therefore, sampling for any kind of analysis, should be decided after considering thoroughly its necessity and the consequences, and above all after considering the ethics behind such a decision.

In this project, particular requirements within the experimental design were the use of a set of non-destructive methods in order to obtain the maximum of information before proceeding to destructive instrumental techniques; to remove the smallest possible size of sample from the icons; and finally to do multiple analyses. This would produce as many reliable results as possible.

1.3 Hypothesis for this research

The history and philosophy of the Byzantine art and all the artistic movements that started from it have been extensively studied (Xygopoulou, 1957; Demus, 1970; Taylor, 1979; Hatzidakis, 1987; Ecco, 1992; Dizirikis, 1996; Andreadi, 1999; Paliouras, 2000; Nicol, 2004). They have been studied in terms of history, art, and philosophy. However, the scientific study of these works of arts and especially the icons is something relatively new. No extensive research has taken place, in order to characterise the materials from which an icon has been made and even less analysis has taken place in the field of the binding media. The analysis of the proteinaceous binders is quite complicated, however, from these analyses, the differences in the painting techniques used through the centuries can be understood, since the binding media have been subject to more changes than the rest of the materials used.

In the history of pictorial art, the Post-Byzantine period could be characterised as a transitional period due to the fact that the binding medium changed from egg yolk to oil and the substrate from wood to canvas. This period and the associated changes could be described as "dark" since little historic or scientific information is available for its understanding. However, its comprehension is of high importance for the study of art both in terms of continuity and as a whole. Extensive literature research showed that there is little work reported on this field. What has been done mainly concerns microscopic examination of paint layers and instrumental analysis of pigments (Bikiaris *et al.,* 1999; Aloupi *et al.*, 2000; Terlixi *et al.*, 2006).

1.3.1 Aims and Objectives

The aim of this project was to provide information on the nature of the binding media used by the post-Byzantine painters from Crete and the islands of Ionion and to study any possible changes, due to Western European influences. More specifically, the aims and objectives of the current thesis were:

Aims

- ✓ To study the effects of western influences on the techniques and painting characteristics of the post-Byzantine icons of Crete and the islands of Ionion.
- ✓ To study any changes in the paint binding media through scientific analyses.
- ✓ To create a technological background (database), which was so far available only from artists' handbooks.

Objectives

- ✓ To provide scientific evidence of how the painting techniques were influenced by western practices, showing how the egg tempera binding medium was slowly replaced by oil, and how the transition was effected through a mixed egg/oil phase.
- ✓ To set up a methodology that would ensure the collection of as much information as possible from each paint sample by starting the analysis with non-destructive methods for obtaining a first set of results. Then to proceed to a destructive method such as GC, which appears to be necessary for the positive identification of egg and drying oil, but with which there is always a risk of loosing the sample.
- ✓ To produce a valuable manuscript of the post-Byzantine icon painting technique that will be used for future reference by scientists, art historians and researchers in general and to provide sufficient information for any future works that might be carried out.

A literature review was carried out to identify all previous work relating to the identification of pigments and binders used in iconography during the period under investigation. Based on the limited information available the following investigation was set up.

In this research samples were collected from different locations along the route of the post-Byzantine art development and from the work of the most important

artists, especially the ones who represent this period but who also contributed to the evolution of the later pictorial art. The samples will therefore provide time/location data slices concerning the creation of the artwork.

In order to achieve these time/location data slices 201 samples were collected from 121 panel paintings, from different chronological periods, exhibited in several museums and churches around Greece (Figure 1.3); for example the Monastery of St John Theologos of the Cycladic island of Patmos, and the Post-Byzantine Museum of the Ionian island of Zakynthos. These samples were used in order to identify the binding media present and to detect the possible presence of oil, which would be evidence that the icon painting technique was indeed influenced by Western European and mainly Italian techniques of panel painting.

Figure 1.3 Map of post-Byzantium. The red circles indicate the areas from which the samples were collected.

Taking all the above-mentioned factors into consideration a protocol was developed using the following sequence of investigations:

- Scanning electron microscopy / energy dispersive X-ray analysis (SEM/EDX) for elemental identification of pigments.
- Fourier transform infrared microscopy (μFT-IR) for pigment identification and detection of the amide linkages for egg, and the triglycerides for oils.
- Raman spectroscopy (RS), as a complementary or comparative method to FTIR for pigment identification and detection of amide functions for proteins and long chain carboxyl groups for drying oils.
- Gas chromatography (GC) for medium identification and verification of the results obtained by the spectroscopic methods.
- Staining of cross-sections for gross-characterisation of the binding media present, in case collection of powder sample is not possible.

To provide reference data for this investigation, a series of samples of different binders – pure oils, egg yolk, animal glue, casein and emulsions – based on traditional Byzantine and Western European methods (Cennini, 1990; ek Fourna, 1906; Doxaras, 1890) were prepared and portions were artificially aged.

In the thesis that follows, the author has tried to put into context the research that took place in order to fulfil the aims and objectives. Chapter 2 presents the historical frame in which the cultural interchanges and artistic changes occurred, indicating why these happened, under what circumstances and the effect they had on the Greek and western European civilisation. Additionally, the materials used by the Byzantine, post-Byzantine and western European artists are presented in an effort to comprehend how the artistic methods and techniques evolved from one style to the other through written sources.

Chapter 3 reviews the analytical methods used for the characterisation of pigments and media. The section is quite important since it presents the work

done during the last twenty years on the physicochemical analyses of paint layers from panel paintings. The degree of research done on icons is extremely limited.

Chapter 4 refers to the experimental methodology and procedure. A very important part of the experimental section is the formation and development of a suitable database that would ease the experiment and the interpretation of the results and that would offer valuable information for the understanding of the materials' mechanisms. Additionally, this chapter includes a reference to the samples taken from post-Byzantine icons along with a short presentation for each one of them (i.e. subject – artist – date – province – location).

In the following chapter 5 the results of the analytical methods are presented and the derivation of the data is explained. In the first part of the section, spreadsheets of the results are being given, whilst on the second part which involves the discussion, the results are analysed in order to draw information, observations and answers about the questions set at the beginning of this research.

Finally, chapter 6 is the conclusion of the thesis. It is the chapter in which conclusions are drawn: to what extent the post-Byzantine artists were affected by Western European art and whether the post-Byzantine art was the transitional period in which the artists passed from egg tempera on wooden panels to oil painting on canvas. At the end of the thesis, recommendations for future work are given.

CHAPTER 2

FROM EAST TO WEST

2.1 Historic Frame

The scope of this section is not to provide in any case a historiographic approach, since it is not the aim of this research and the subject of the author. On the contrary, the aim is to provide the historic frame in which the main question of this research stands. The historic events, as well as the social and political context of the time, help the author understand the route the art and the artists in question followed. This chapter will try to explain the changes from one style to the other and the transitional period in which those changes took place (Figure 2.1).

Figure 2.1 The two different styles are distinct. A) is quite close to the Byzantine prototypes, while B) has incorporated the western influences and resembles a painting more than an icon.

A) *St Nikolaos* by Paleokapas, egg tempera on wooden panel, 1637

B) St Gregory the Theologian by Kantounis, oil painting on wooden panel, beginning of 19th c

The fall of Constantinople marked the end of a long history and the end of a great civilisation. The magnificent capital was built by Constantine the Great on the coast of Bosporus, where centuries ago, Byzantas had established the homonymous colony. For more than a thousand years, Constantinople was the richest city of Christianity, with industries, workshops, busy markets, palaces, massive walls and more than 400 churches. Within the darkness of the Middle Ages, the Eastern Roman Empire was the earthly expression of the heavenly kingdom of God. The Byzantines, with a deep religiousness and faith as their guide, had the feeling that it was their historic duty to resist the decadence and the insecurity of the Middle Ages. They had the duty to retain as much as they could of the classical civilisations of the past, Greek and Roman. Greece, Rome, Christianity was the fundamental trilogy on which the Byzantine civilisation was based (Garoufalis, 2002).

Byzantium may have lost its political existence with the conquest of its capital by the Ottomans in the fifteenth century, but the Byzantine civilisation continued its existence during the following centuries. As Runciman (1969) mentions: "If a proof for the existence of the Greek history is necessary, then this comes from the rebirth of the interest on the classic writers, which is repeated during the whole duration of the Byzantine history".

2.1.1 Byzantium

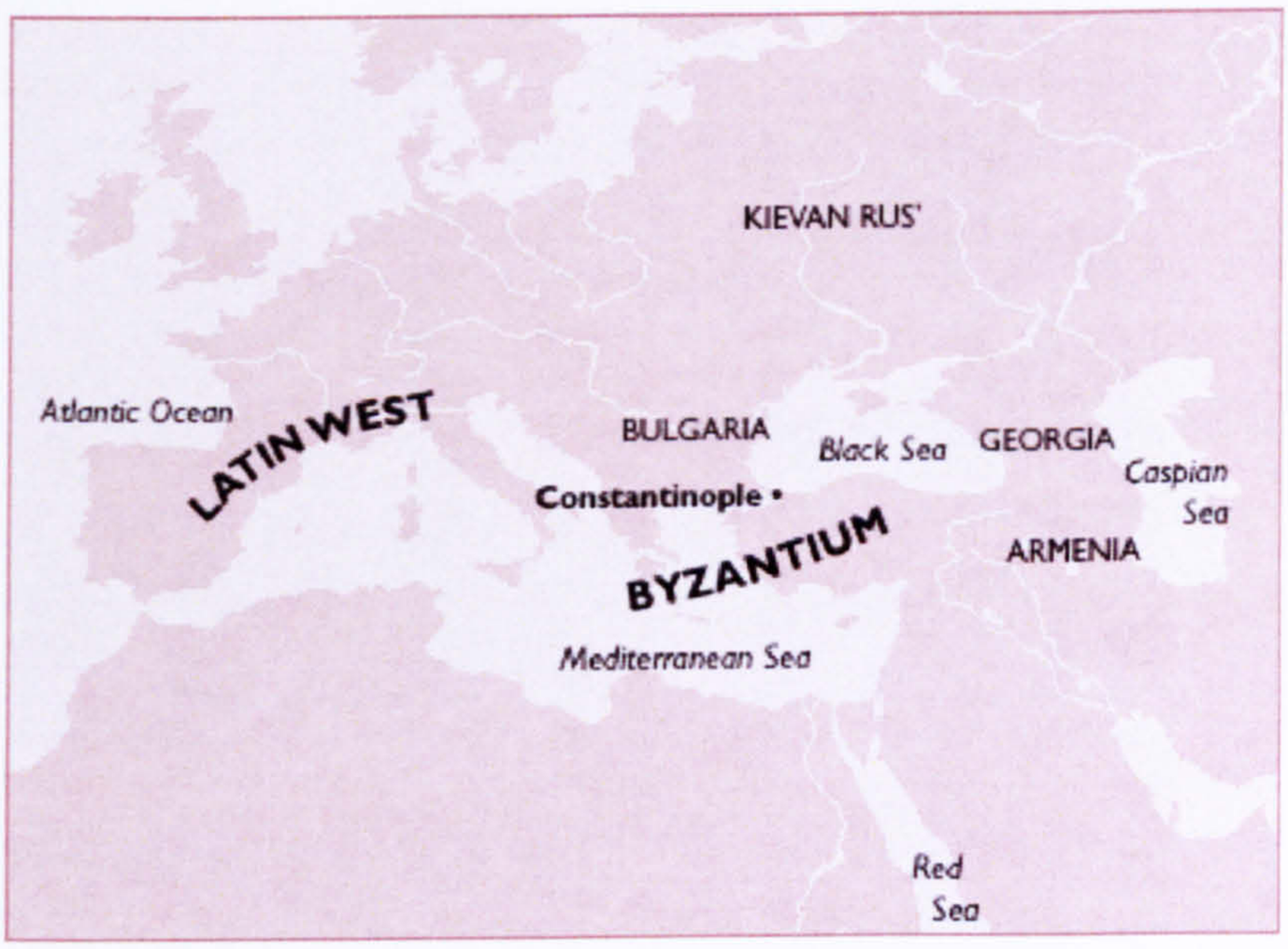
2.1.1.1 Social and political context

At the end of the 3rd century, the administration and economy of the Roman Empire was in such a great chaos that there was a danger for the Empire to fall apart (Guillou, 1996). The need for transformation was inevitable, along with the need for a new administrative centre.

In 323 AD Constantine the Great becomes the new Emperor. In the meantime, the 1st Ecumenical Synod established the principles of the Christian dogma and elected the Emperor as head of the Church.

In 324 AD Constantine the Great decided to transfer the centre from Rome to the old city of Byzantium. The choice was based on the strategic location of the city. It was located at the very edge of Europe, in Bosporus, one of the two most commercial crossroads of history, the easiest and more approachable passage between Europe and Asia. On May the 11th of 330, the Emperor inaugurated the city with the name New Rome, or as its people preferred to call it by the name of its founder, Constantinople. However, this capital, which was from the beginning a Christian capital was going to change totally the scene in the Roman matters. The Roman Empire was going to be divided into two areas: the Western and the Eastern Roman Empire. This led eventually to the formation of the Byzantine Empire (Figure 2.2).

Figure 2.2 Map showing the Byzantine Empire and the Latin West



The long duration of the Byzantine Empire was due to the constitution and the administration regime (Runciman, 1969). An indispensable basis of the society was an inborn respect of the law, and the adoration of the state and the emperor. The regime was authoritarian monarchy. The Empire had a very well organised structure at all levels; a strong army and navy; an excellent educational system, a flourishing trade and an important artistic production. Furthermore, the level of the civilisation was remarkably high. As far as

languages were concerned, the empire was divided into two. All the inhabitants of the western provinces spoke Latin, while those of the eastern ones were speaking Greek. This division seemed to be more superficial than real, since all the educated people followed the prototypes of the Greek world. Last but not least, religion played a very determinant role. It is important to underline, how insignificant life in this world was for the Byzantines. Even though Christianity was developed in a difficult and dark period, the promise of a better afterlife gave the masses the chance of an escape from the present life. Christianity ruled the lives of the people and it also played a big part in the administration of the state through the Ecumenical Synods, whose decisions were respected by all Christians.

2.1.1.2 Trade

According to Runciman (1969) the history of Byzantium was first of all the history of the economic policy and the history of trade of the Middle Ages. As it was mentioned above, the location of Constantinople was in the crossroad between East and West. It was almost inevitable for people and merchandise that travelled from one continent to the other, to pass through the city. Likewise, ships crossing the Black Sea and Aegean Sea, could not avoid docking there. During those times, Far Eastern trade was the main commercial trade of the world. Byzantium became the crossroad for the import, manufacture and trade of commodities to Western Europe.

2.1.1.3 Art

The Byzantine art was mainly a religious art in all aspects. It was a means to lead, educate and inspire the faithful. A few pages are not enough to describe the entire artistic movement that started and developed during this era. Monumental art, mosaics, mural paintings, manuscript illumination, even music and hymns were among the sections of art which fully developed, met the demands and changed the standards of the time. However, the most important contribution of Byzantium to the artistic world was icon painting.

Iconography followed strictly the orthodox dogma and depicted with sacred devotion the holy manuscripts (Taylor, 1979), thus it tended to remain fairly static. Since it was a mere depiction of the Holy Bible and the Gospels, no changes were allowed, unless they were following changes in theological ideas. The icon painter, was purely the intermediary between God and the humans, therefore only a particularly fine interpretation of the style of a given scene could be seen as an especially blessed creation.

The style was a uniform mixture of Greek, Roman and eastern elements (Talabot, 1965). With the roots to the ancient Greek naturalism and the Fayoum portraits (Doxiadis, 1995; Kouloumpi, 2000), along with elements of paganism and Christianity, icon art developed a unique character inspired from a deep, transcendent, almost mystical symbolism.

The forms are simple, the two-dimensional landscape lacks of depth, and the figures carry the weight of the "passion". Big eyes, long noses, strong lines, frontal positions, lack of perspective, are only some of the characteristics.

The iconoclastic movement of the 8th century prevented the icon painting art from developing and evolving further. During this period, icons lost the Empire's protection and became the clandestine subject of the persecuted Christians. It took more than a century for the icons to be restored to the Orthodox worship. After the restoration, icon painting art reached its climax. The drawing is of high quality, the figures gain elegance, individuality and movement.

A lot of those icons were executed by monks, since it was their duty to transfer the word of God to the believers. This is the reason why most of the early icons are not signed and they are called aheiropoiites (not made by human hands) (Kontoglou, 1979). As the art was developing through the centuries, icon painting acquired more of an artistic character and painters with their workshops took on the execution of the panel paintings.

2.1.1.4 East and West

The Byzantine ideas influenced both eastern and western world. Islam was among the receivers of the Byzantine culture. On the other hand, Western Europe received the treasures of the Classic Philosophy, the artistic trends, the literature, along with the wisdom which was going to affect the Renaissance (Dizikirikis, 1996). Byzantine icon painting gave the percept of envisagement thus creating strong sentiments. Then again, Renaissance suggested a complex form of respectful and imaginative art, with strong faith on the earthen life.

The Venetian art, especially, was directly affected by the Byzantine one. These influences affected the other European countries as well. The German architecture incorporated Byzantine elements in its rich decoration; indirect influences to the French art through the Venetian prototypes; few influences to the English art as well. The 16th century critic and biographer Giorgio Vasari uses the term *Maniera Greca* (Greek manner) in order to describe the 13th century panel paintings made in a style influenced by the Byzantine art (Andreadi, 1999).

The interactions with Venice were very immediate. Even though Venice was created as a province of the Byzantine Empire, with time it became an ally and associate. When the Byzantine world started falling apart, it ended possessing vast colonial acquisitions in the internal of the decomposed Empire (Nicol, 2004). Additionally, Venice could be described as an intermediary between East and West, due to the fact that it had the most important port of the West and commercial relations with Constantinople. There was always a commercial delegacy in Constantinople, while young Italian people were going to the Byzantine capital to study.

Unfortunately, the relationship between the two representatives of East and West, Constantinople and Rome, proved to be problematic. From one side the transfer of the capital and from the other side the antagonism led the two

pontiffs in 1054 AD to excommunicate each other, creating not only a religious schism, but a de facto schism as well.

2.1.1.5 Crusades

The Crusades were basically the beginning of the end of the Byzantine Empire. The 11th century was the century of the Christian awakening of the believers of the west and the rise of the movement towards the East (Runciman, 2006). To the question why the Crusades started, a simple answer could not be given. The idea of the Christian holy war is the central point of this movement. The "war of justice" towards the defence and recapture of a legal possession could be the answer. The recapturing of Jerusalem from occupation by the non-Christians, transformed many Christians into warriors in order to chasten their sins and gain the eternal salvation of the soul. However, behind this idea, the remote truth was the demographic expansion, the provision of safer trade routes and the capture of Constantinople (Berstein and Milza, 1997).

Four crusades took place (1095-1204). The fourth one was the most catastrophic of all and it was the one which turned against Constantinople. Even though the Crusades had originated in a call for aid by the Byzantine emperor Alexios I Komnenos for the defence of the Empire, yet in 1204 the soldiers of the Fourth Crusade invaded Constantinople and dismantled Byzantium (Figure 2.3). The perfect conditions for the Ottoman invasion had been created.

Figure 2.3
The Entry of the Crusaders into Constantinople, by Eugène Delacroix, 1840

Venice was the big winner of this catastrophe. Venice took the Greek islands and established colonies on the coastline, while securing the exclusiveness of the eastern trade. Venetian colonies were scattered in the eastern Mediterranean, the Aegean Sea and the Black Sea. Difficult times had started for the Byzantines, with short good periods.

The end of the Empire came on the 29th of May 1453. Constantinople was sacked for 7 weeks by the Ottomans.

2.1.2 Post-Byzantium

2.1.2.1 Social and political context

After the fall of Constantinople, the Ottoman threat led the Greeks to abandon their lands and properties and to ask for asylum in Western Europe, the non-Ottoman invaded Greek lands and the Venetian colonies. The collapse of the Byzantine Empire created a wave of migration towards the west. A large number of Greeks moved to Venice where they managed to integrate, while keeping their national identity. In 1498 the *Scuola* was established, a national minority fraternity in the heart of Venice, which played a very important role in the spiritual life of the city-state (Nicol, 2004).

In the meantime, Venice, either with direct or indirect conquests of the Greek lands, managed to rule the fate of the Hellenic world. It was more of a federal state with very well selected Greek colonies at very strategic crossroads. In these colonies, the rich Byzantine culture came in contact with the trends of the Renaissance in Europe, thus developing a unique blend of both artistic influences.

Crete and the islands of Ionion were the Greek areas which presented the highest artistic action, giving birth and evolution of the Post-Byzantine School. This study will focus on those islands only.

2.1.2.2 Crete

The geographical position of Crete, along with the Cretan temper, contributed to the political and artistic history of the island, which could be described as "adventurous" (Clogg, 2002). Initially part of the Roman Empire, and later part of Byzantium, it was quite often the main target of the pirates and suffered from the Arabs, whose conquest lasted for 133 years (888 – 961 BC). In 1204 Crete became a colony of the Serene Republic of Venice. The Venetian Rule lasted for four centuries, until 1669, when the Ottomans managed to invade the island.

Crete occupied the most important role on the map of the Venetian colonies in Greece. The strategic position of the island at the significant crossroad of the trade routes offered Venice the naval domination in the Eastern Mediterranean.

2.1.2.2.1 Art and artists

After the capture of Constantinople, a stream of refugee painters, among them very important ones, moved to Crete, and changed the artistic standards, which until then were rather provincial.

According to the Venetian archives, between 1453 and 1526 in the capital of Crete, Candia, the number of painters had reached 120. Since Crete was under the Venetian Rule and the citizens of the island were both Greek and Venetian, the icon painters had to produce panel paintings for a geographically, religiously and ethnically differentiated clientele, with various artistic preferences. So, they were trained to paint in different styles (maniera Greca & maniera Italiana - Greek way & Italian way) in order to satisfy all tastes (Hatzidakis, 1987). Additionally, the portable icons became objects for export to all the Christian communities, i.e. Western Europe, Russia, and Asia.

The emergence of the Cretan School of icon painting changed the scene in the art world. It became the most important and high quality painting school of the time and produced a large number of excellent painters (Appendix I). Cretan art was initially a continuation of the Byzantine style. With time, it adopted western

characteristics, mainly through the Italian influences (Venetians in Greece and Greeks in Venice). As a general rule, one could say that the Cretan School of icon painting kept the Byzantine characteristics, while it tried to integrate achievements of the western art without violating the Byzantine heritage (Demo, 1970).

A remarkable effect of the renaissance influence was the social ascent and improved educational background of the painters. Additionally, as previously mentioned, the icons in the Byzantine period were not considered as artefacts or made by human hands, thus they were not signed. In Crete, the artists started signing their artefacts (Figure 2.4), since the icons acquired the role of a painting than of a mere narration of the Bible (Demo, 1970).

Figure 2.4 Cretan icon of the Virgin Mary the Vatos, made in the 16th century by M. Damaskenos. At the bottom left hand side of the painting is the signature of the painter: *Ποίημα Μιχαήλ Δαμασκηνού (made by Michael Damaskenos).*

2.1.2.3 The Ionian Islands

The Ionian islands remained under the Venetian Rule for around four centuries, which was enough time to determine the character of the islands and their people. While the rest of Greece was under the Turkish yoke, the citizens of the Ionian Islands were enjoying the feudal system, the new social classes of the nobles, the bourgeoisie (civili) and the popolari (common people) and the flourishing of the intellectual and artistic life (Paliouras, 2000). The fact that the Greeks and the Venetians had so-well integrated into one body helped those islands to flourish in all aspects.

The Venetian presence in the Ionian islands lasted up to 1797. After that the Ionian Islands returned back to the Frankish Rule, later passed on to the Russian Rule. In 1814 they were declared an independent state under the protection of Great Britain and in 1864 they regained Greek identity.

In Corfu, the Venetian Rule was established in 1386; in Zakynthos in 1483 and in Cephalonia in 1500; the smaller islands of Ithaca and Paxoi appended in Cephalonia and Corfu respectively. Kythera became part of the Venetian colonies in 1363, and Lefkas in 1699.

2.1.2.3.1 Art and artists

The end of the Venetian Rule in Crete in 1669 by the Turkish invasion was the end of the Cretan School and the emergence of the School of Ionion or Heptanesian (seven islands). All the artists who lived and created in Crete migrated to the Ionian islands. However, the painting school (Heptanesian School) which emerged from this historic event, never equaled the glory or the quality of the Cretan school.

As it was previously mentioned, the Cretan School kept the Byzantine characteristics, while it tried to integrate achievements of the western art without violating the Byzantine heritage. On the contrary, the Ionian School adopted the

western characteristics and moved a step further, setting itself free from the Byzantine strict rules of depiction (Kontoglou, 1979).

Gradually, the painters tried to depict the themes naturally and realistically and to add perspective to the scenes. With time, the icon painters dared to adopt into their composition renaissance themes as they were, resulting into paintings which presented Biblical themes developing into landscapes (Dizikirikis G., 1996).

Panagiotis Doxaras (1662-1729), as a theoretician, could be described as one of the forerunners of the Greek Enlightment and as an artist the one who, without disregarding tradition, defined the new framework in which at least four generations of painters in the Ionian School were to operate. His treatise Περί Ζωγραφίας (About painting- a translation of Italian texts about art and painting), was considered as the definitive written document on the establishment and beginning of a new school, the Italian-Greek (Votokopoulos, 1990). The trend that followed his work produced a large number of important paintings which with time moved from the Italian influences and developed a unique style leading to the beginning of the Neohellenic art of the 19th century.

2.2 Artists' materials and techniques

Painting, as a means of human expression, goes back to prehistory. According to Albenda (1970) "it was employed to depict images and symbols, usually of a religious nature, and to record events pertaining to daily life". Initially, the means by which the artists recorded their subjects were simple, using natural materials easily found in nature, as simple were their forms of depictions. Gradually, they became more aware of the materials and their properties and they started evolving specific techniques.

One of the main paint media of the ancient world was egg yolk (Albenda, 1970). This was used not only for the construction of paintings, but also for mural paintings and manuscripts. This is the medium inherited in the Roman and

Byzantine world. However, it was not until the Byzantine Empire that the art of egg tempera typified as a technique and adopted from both the Eastern and the Western world. Italian masters such as Giotto, Simone Martini, Michelangelo, etc. used egg tempera to produce a great number of masterpieces.

Renaissance and the whole philosophical trend of human liberation and rebirth behind it, was the period in which the masters started experimenting with other media. It was also the period in which the artists introduced popular subjects apart from religious ones. At this point, Flanders was about to play the most important role. In circa 1410 Jan van Eyck (1390-1441) was the master who *invented*[1] oil as a medium on wood panel (Vasari, 1998). Oily materials begun to be introduced into the tempera technique, mainly by the Venetian artists of the 15th - 16th century, creating thus another technique, the *tempera grassa*. Oil gave another perception in the art of painting and gradually in the 17th century, replaced egg tempera on wood panel. Oil painting on canvas was adopted. However, the Hellenic world was going to follow that change a couple of centuries later (late 18th century).

2.2.1 Byzantine techniques and materials

Icons are panel paintings of religious context, which originated from Christian Orthodox countries. The earliest icons that have survived come from the 6th century but it is said that pictorial representations of Jesus Christ and His Disciples were painted when they were still alive. According to Talbot, Rice (1963), the art of Byzantine icon painting is a continuation of the Hellenistic tradition and the Egyptian Fayoum portraits.

Due to illiteracy, icons had a self-explanatory character but their role was spiritual. Standardisation came in order to give icons a mystic and underworld character, for the representation of a "translucent" and non-material world. Gradually, the representation of religious subjects was made by following strict

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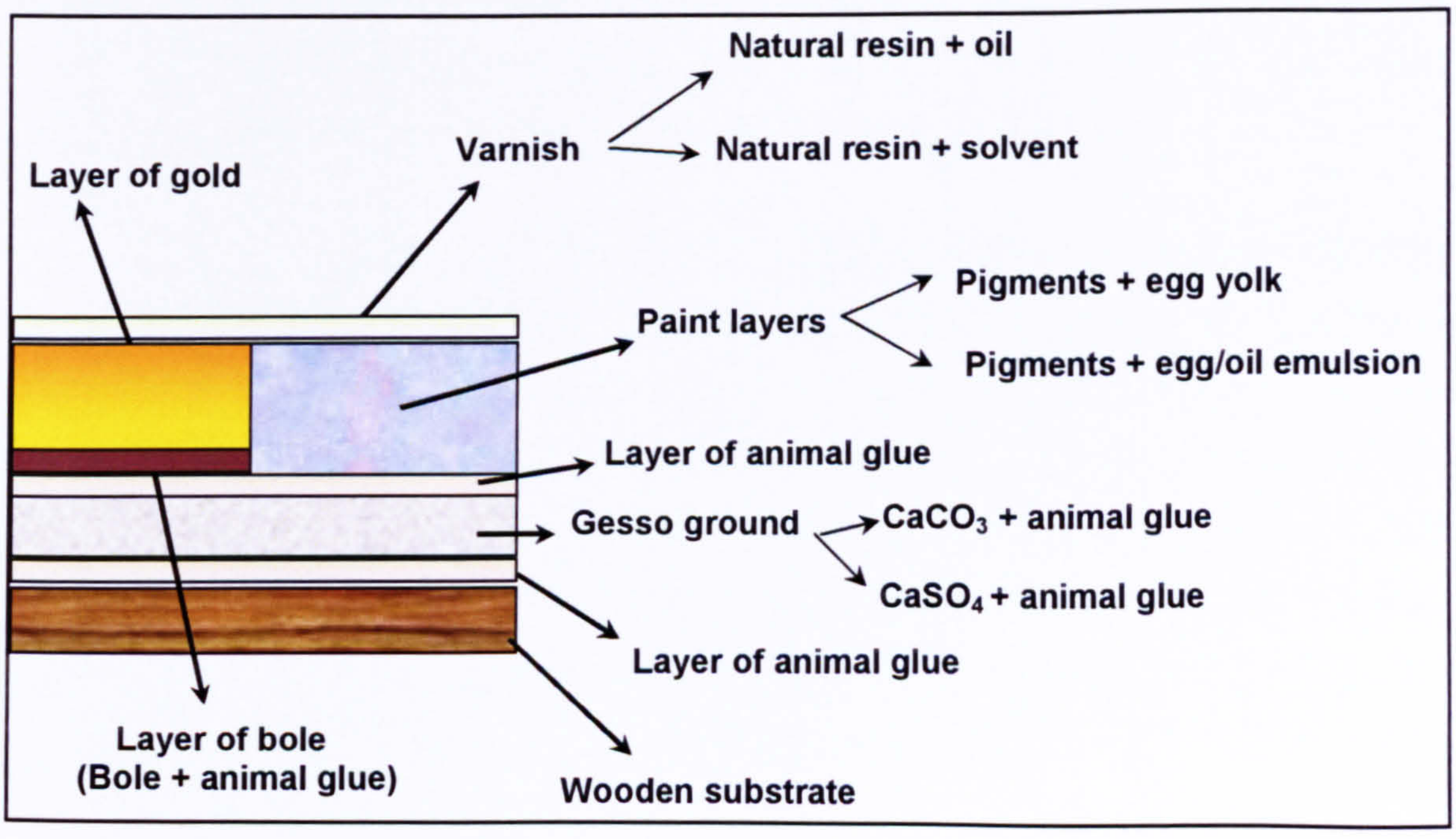
[1] or re-invented, since Theophilus, a German monk of the 12th century, refers to the use of oil in the painting

rules. Dionysios ek Fourna, was an early 17th century icon painter and author of the treatise "Ερμηνεία της Ζωγραφικής Τέχνης" (the interpretation of the art of painting), in which he fully described the pure Byzantine icon art.

During the construction process (Taylor, 1979), four main constituents were used (Figure 2.5):

- **The substrate**, which is usually softwood (pine, cedar, etc) often covered with linen to prevent the consequent layers from cracking.
- **The ground preparation layer**, or gesso, which is calcium carbonate ($CaCO_3$) or calcium sulphate ($CaSO_4$), mixed with animal glue.
- **The paint layer**, which consists of grains of pigment of the desired colour mixed with a medium. According to the 17th century Greek artist and author of a treatise that describes the Byzantine technique, Dionysios ek Fourna (1906), this medium was hen's egg yolk. Egg yolk gives the pigments a shiny, dark and semi-transparent colour. Egg tempera is the mixture of egg yolk, with vinegar and water.
- **The varnish** is the last constituent of the icon. Its role is to protect the paint layer and to give the sense of uniformity to the surface.

Figure 2.5 Cross-section of an icon



The painting process was as follows (Vickrey and Cochrane, 1973; Mayer, 1991): the eggs were cracked into the palm of the hand; the egg white was removed by washing with water; the skin of the yolk was pinched and the contents emptied into a bowl; the seed was removed; finally, the egg yolk was mixed with vinegar. Some artists added the pigments in the mixture or they grounded the pigments up, mixed them with water and then mixed the pigment paste with the medium. The addition of vinegar was to reduce the greasiness of the egg yolk and to act as a preservative for the egg tempera. The amount of vinegar added, was reduced gradually from equal volume to the egg to 2-3 drops.

The amount of medium added to the pigment depended on the nature of the pigment and on the final effect that the artist was trying to achieve. However, old schools of painting used to mix the pigment with a strong egg medium in order to harden the paint layer and give the effect of enamel.

2.2.2 Post – Byzantine techniques and materials

Unfortunately, there is not enough literature on the techniques used by the Post-Byzantine artists. The main sources have been the treatises of Panayotis Doxaras (1662-1729). This famous Post-Byzantine painter was very deeply affected by the Italian painting and especially the Venetian culture. He wanted to spread among the Greek artists, the principles of the "perfect and exact painting", which were based on the naturalistic form of painting.

Therefore, he translated the handbook of painting, written by Leonardo da Vinci (1459-1472) and other art texts written by Leon Batista Alberti (1404-1472) and Fra Andrea Pozzo (1642-1709). However, his major contribution was his personal treatise "Περί Ζωγραφίας" (about painting), written possibly around 1725. In this document, methods and materials of this era, along with personal ideas were described. He mainly referred (Doxaras, 1871) to the oil painting, giving advice about the drawing and recipes for oil varnishes, oil painting, etc. His aim was to convince all his contemporary artists to accept the technical evolution of painting.

2.2.3 Western European techniques and materials

The main sources of information regarding the techniques and materials used in the period of the Renaissance in the west and specifically in Italy are Cennino Cennini and Giorgio Vasari. In 1437, Cennini, an author and artist, student of Angolo who was a student of Giotto, completed a treatise in which he described in detail the methods the artists of his time used in order to produce their artefacts.

According to Cennino Cennini (1954), the Italian panel painting technique of that period involved more or less the same procedures used by the Byzantines, but the pigments' medium differs. For the egg yolk he says: *"the tempera consists of the yellow of the egg. Know that it is widespread."* He also presented a painting procedure integrating tempera painting covered by light oily layers. *"...with cinnabar, which should have egg yolk as the glue; then, with an oil lacquer we should apply one or two layers on top..."*. Vasari, an author and critic of the 16th century wrote about the lives and the art of the famous artists, describing also their techniques. In his treatise he mentioned the use of egg yolk, drying oils and emulsions.

According to Mayer (1991) egg/oil emulsions were used in the western European art by mixing egg yolk with stand oil, sun-thickened oil or cold-pressed linseed oil, while the most commonly used drying oils where linseed oil, poppy oil or walnut oil.

2.2.4 Chemistry of the main pigments and binding media

The structure of a panel painting is quite complicated, consisting of subsequent layers of gesso ground, painting and varnish onto a wooden substrate. Each of those layers, apart from the varnish, comprise mostly of a mixture of organic binders (egg yolk, drying oils, animal glue, casein, etc) and inorganic components (inorganic pigments, gypsum, etc).

2.2.4.1 Binding media

The organic binding media solidify through polymerisation or oxidation. When they are in the liquid state, they act as dispersive media of the pigments for the formation of a homogenous mass, which is called media. However, when solidification occurs, the pigment particles are held firmly in position and they are then called binding media.

2.2.4.1.1 Egg Tempera

The term "tempera" is used for every aqueous medium employed to drench the pigment powders and make then usable in painting. Egg yolk is a natural emulsion consisting of proteins, lipids and water.

The composition of a hen's egg (Phoenix, 1997; Mills and White, 1994) is shown in the following tables (2.1 – 2.3):

Table 2.1 The composition of hen's egg

Percentage of constituents (% p.w.)	*Egg yolk*	*White*
Water	49	87
Proteins	16	10.5
Triglycerides	22	-
Phospholipids	10	-
Cholesterol	1.5	-
Glycerides	0.4	0.6
Inorganic components	1.1	0.7

Table 2.2 The fatty acids present in egg yolk

Fatty acids
14 :0*
16 :0
16 :1
18 :0
18 :1
18 :2
18 :3
20 :4
22 :5
22 :6

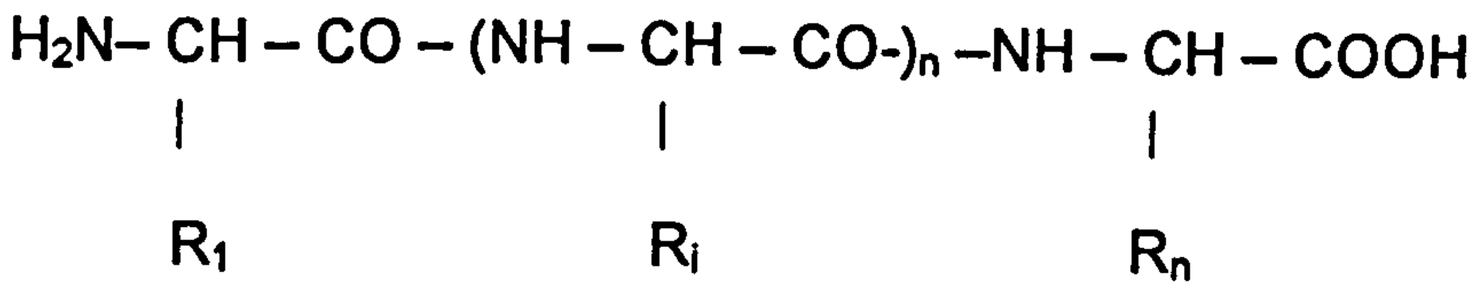
**the first number represents the number of carbons present while the second number represents the number of bonds present*

Table 2.3 Analysis of the amino acids (%) present in egg yolk by Keck and Peters (1969)

Amino acids	*%*	*Amino acids*	*%*
Glycine	*6*	Serine	*11*
Alanine	*8*	Threonine	*6*
Valine	*7*	Cystine	*2*
Leucine	*9*	Methionine	*2*
Isoleucine	*5*	Arginine	*4*
Proline	*5*	Histidine	*2*
Phenylalanine	*3*	Lysine	*5*
Tyrosine	*2*	Aspartic Acid	*11*
		Glutamic Acid	*13*

Protein (Figure 2.6) materials, such as hen's egg, are made up of a variety of building blocks, known as amino acids. These are nitrogen-containing acids, which can be aliphatic compounds, branched chain aliphatic, aromatic and heterocyclic.

Figure 2.6 Polypeptide chain

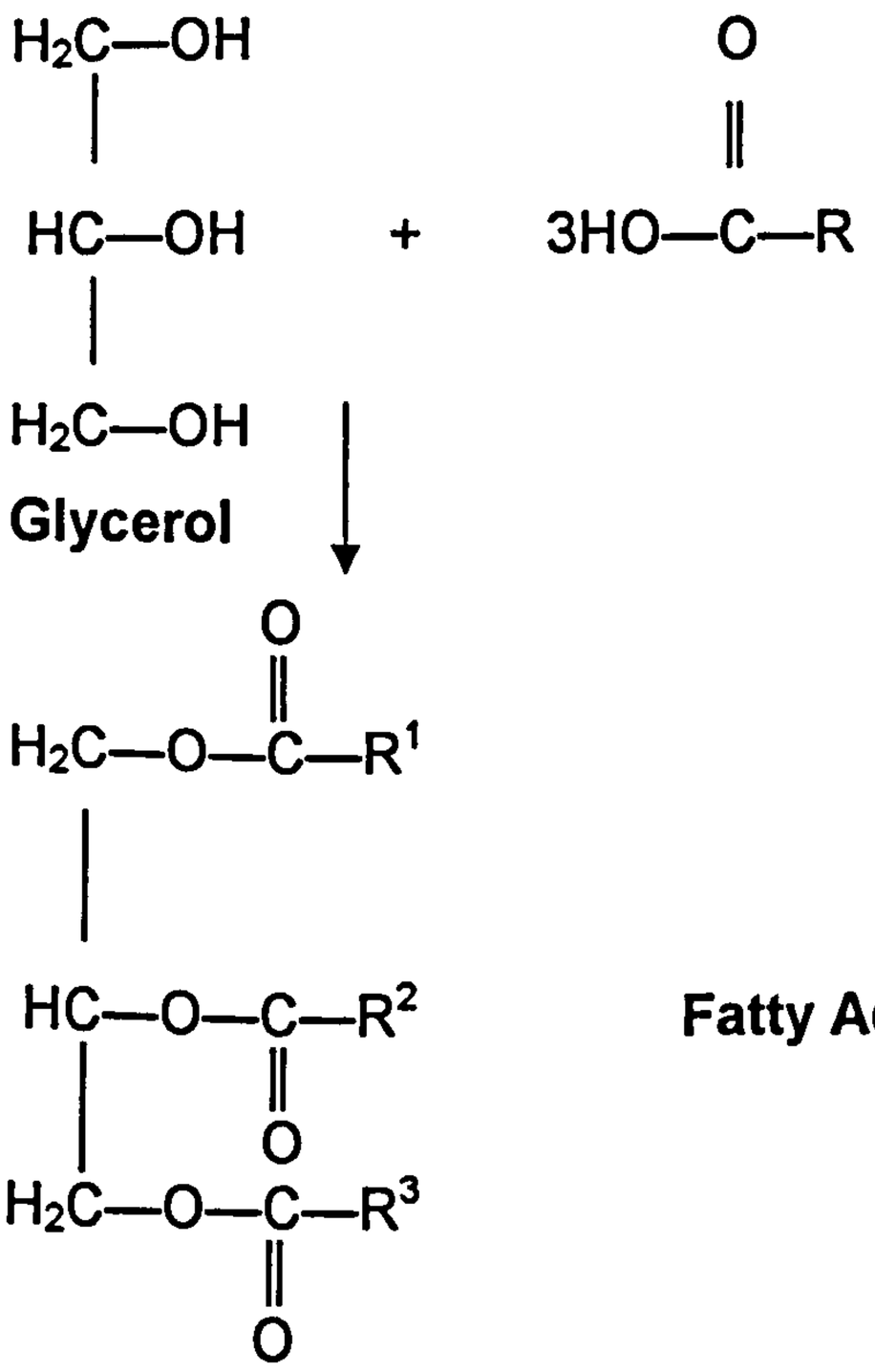


According to Phoenix (1997), when egg tempera dries and solidifies, through the evaporation of the water and the oxidative polymerisation of its lipid components, it forms a hard and durable film. Its properties are very much determined by lipid and protein components and lead to cross-linking. Metal ions from pigments may affect curing processes and lead to deterioration of the medium. For example, Hg from cinnabar accelerates cross-linking. The molecular weight of protein increases with ageing through polymerising and cross-linking, which also results in a 25% reduction in free amino-acids content.

2.2.4.1.2 Drying oils

According to Mills and White (1994), oils are mixtures of mixed triglycerides, which are the esters of glycerol with a wide range of fatty acids. Most fatty acids are linear, long carbon chains containing 18 carbon atoms (Figure 2.7). The polymerisation of oils occurs by oxidative cross-linking. This process is initiated by the double bond present, so the more double bonds there are in the fatty acid, the more reactive the acid is.

Figure 2.7 Typical form of a fatty acid



When oils are applied as thin coatings, they absorb atmospheric oxygen and polymerise, forming a tough, elastic layer. During polymerisation, degradation products, such as aldehydes and carboxylic acids are produced. After drying, the oils cross-link and they become insoluble with increased density and refractive index. Ageing of the mixture results in progressive formation of dicarboxylic acids such as pimelic, suberic, azelaic, and sebacic acids which are mainly found in old paints (Surowiec et al., 2004).

All the oils used in painting are mainly vegetable oils (Table 2.4). The ability of some oils to dry permits the painting with this material. For example, soy oil, cottonseed oil and sesame oil dry very slowly. Linseed, walnut, and poppy seed oil dry quickly, while olive and hazelnut oil do not dry at all. European painters used mostly linseed, walnut, and poppyseed oil (Dionysios ek Fourna, 1903; Cennini, 1954; Mayer, 1991). Hempseed, tung oil, perilla oil and sunflower oil, among others, have been also mentioned in the early literature (Pliny, 1988).

Table 2.4 Fatty Acid Percent Content of Oils & Hen's Egg according to Mills & White (1969):

Organic media	*14:0*	*16:0*	*18:0*	*18:1*	*18:2*	*18:3*	
Linseed Oil	Tr.	6 - 7	3 - 6	14 - 24	14 - 19	48 - 60	Tr.
Poppy Oil		10	2	11	72	5	
Walnut Oil		3 – 7	0.5 - 3	9 - 30	57 - 76	2 - 16	
Hen's Egg	Tr.	27	9	44	13.5	48 - 60	Tr.

2.2.4.1.2.1 Linseed oil

Linum usitatissimum L.

Linseed oil is a yellow, drying oil, which comes from the seed of flax (Linum usitatissimum L.). It is one of the main and most important oils used in art due to its good qualities. It is known to dry from the top down after forming a skin, and therefore takes many months before it is completely dry. It contains, mainly, Linolenic Acid, which is an unsaturated fatty acid, and Linoleic Acid, which creates the yellowing, but also give a high degree of flexibility. In order to enhance the good properties of linseed oil, a variety of procedures were being used and a series of modified forms of linseed oil were produced.

Cold-pressed linseed oil was pressed from the flaxseeds without the use of heat or solvents. It is recommended when grinding dry pigment to make oil paint.

Stand oil was linseed oil of increased viscosity, which was acquired through heating the oil to about 315° C under conditions that exclude oxygen. It dries slowly, yellows less and forms a tough durable film.

Boiled Linseed Oil was altered with the addition of chemical drying accelerators, i.e. solvents and siccative such as metal salts. Metal salts could act either as "surface driers" because they aid in the drying of the film on the surface, or "through driers" because they catalyse throughout the film (Tumosa and Mecklenburg, 2004). Sometimes, boiled linseed oil was mixed with 10-15% stand oil, in order to produce a quick drying and elastic oil.

Sun-bleached oil is a type of linseed oil dating back to the 14th century used to produce a fast drying oil, by mixing it with equal parts of water, or sea water, placing it into glass vials and exposing it to the sun for several weeks.

Sun-thickened oil was produced by a further step from the sun-bleached oil. The process was the same, the difference being that the mixture was left into the glass vial for 3-4 months and it was also exposed to air in order to oxidize. It became more viscous and produced a tough, enamel-like, non-yellowing film.

2.2.4.1.2.2 Walnut oil

Walnut oil comes from the well-matured nuts of juglans regia through cold-pressure. The oil obtained is a very fine liquid, suitable for mixing with fine pigments and producing very thin line painting. Even though it dries faster than linseed oil and yellows less, its main disadvantage is that it cannot be stored because it tangs and it has a high cost of production. Walnut oil was used quite a lot by the old masters (Vasari, 1998), especially during the Renaissance. As it has been referred in the art treatises, this oil had better properties than linseed oil. Walnut oil can also be modified as linseed oil, in order to alter its properties. Bleaching under the sun and air, makes the oil dry faster and keep its colour hue stable.

2.2.4.1.2.3 Poppyseed oil


pigments + oil

Poppy oil comes from the seeds of the white poppy (Papaver Somniferum) through cold or hot pressure. Only the mature seeds give good quality oil. Poppyseed oil has lower drying ability than linseed oil. Due to the slow drying, it is not suitable for thick layers of painting, because it cracks. The absence of linolenic acid contributes to the low degree of yellowing. This property gives the oil a light colour and this makes it suitable for grinding whites and blues and using it as a medium for all pale colours. However, the absence of linolenic acid also offers the brittleness of the poppyseed oil film during ageing. These properties make it more suitable for single-medium techniques than emulsions.

2.2.4.1.3 Emulsions

The emulsions are made by mixing the proteinaceous binding media with drying oils or natural resins. Reference to those media has been made by artists who describe the techniques of the great masters of the Post-Byzantine and Italian art, such as Giorgio Vasari, Cennino Cennini and Dionysios ek Fourna.

Egg yolk has the property to emulsify big quantities from other binding media, such as drying oils, solutions of natural resins or waxes, due to its high content of lecithin.

Several proportions of the media involved have been reported in literature (Mayer, 1991). For example: Egg yolk – water – linseed oil 1:2:1.

2.2.4.2 Pigments

Pigments (Feller, 1985; Harley, 1970), are very finely grained (over 0.1 microns across) materials, which when mixed with the medium, disperse into it. The use of the medium is necessary to keep the pigment particles together. Pigments

come from a wide range of substances that can be organic, inorganic, natural, or synthetic. In a medium, they scatter light, and therefore seen as opaque. However, very finely ground pigments, may also allow light to pass through the film and therefore seen as semi-translucent. Generally, the size and the shape of the particles are very important for the homogeneity and easiness of application.

Pigments present chemical stability and inertness towards other chemical substances of the paint layer. It is very rare that two different pigments will interact with each other. It is only possible, under specific circumstances, for mixtures of pigments that contain sulphur, and copper or lead to chemically react and discolour.

Pigments can be quite sensitive to environmental conditions, especially, temperature and oxygen. Light can be a very important cause for chemical alteration, which might lead to change of colour.

The Byzantine icon palette was very specific, limited and strict as the whole philosophy behind it. The Post-Byzantine palette adopted gradually the colours used in Western Europe, which was a more liberated and bold palette with brilliant colours and a vast variety of hues (Feller, 1985). However, the three common pigments used in all three periods of art were lead white, cinnabar, and red lead.

2.2.4.2.1 Cinnabar

Cinnabar or vermillion, HgS (Harley, 1970; Gettens, *et al.*, 1966) was known to Greeks and Romans since the 4th century AD, while in Asia, it became known in the 6th century AD. In Europe it was used until the 19th century. It is a pigment of inorganic, mineral and synthetic origin, which was found in China, Spain, Italy, Germany and Yugoslavia. According to Pliny (1988), the best quality came from Sisopo, Spain.

In nature, it comes as a mineral, which is then treated to produce the pigment. There are two methods of production. The first one is the "dry" method, where mercury is mixed with sulphur, heated up and ground up to produce cinnabar. The second one is the "wet" method, where five parts of mercury are mixed with one part of sulphur in a concentrated solution of KOH and then the mixture is heated up, until a very intense red is obtained. Impurities found are quartz, pyrites, marsacites and stibnite. Quite often cinnabar is mixed with minium (Cennini, 1954).

Cinnabar has the tendency to darken, especially when exposed to sunlight. It decomposes in acids and it alters when mixed with lead pigments. It has very good opacity and covering power. It can be used for tempera, oils, watercolours and frescoes. Its use is not advised for encaustic.

Chemical Name: Mercuric Sulphide

Crystal Formula: Trigonal – Trapezohedral

Refractive Index: 3

Grain Size: Medium, 1-10 microns

2.2.4.2.2 Lead White

In icon painting, white is the basis of all colours. Each pigment can be mixed with white to reach a desired hue. Lead White (Harley, 1970), $Pb(CO_3)_2 \cdot 2Pb(OH)_2$, is one of the most important pigments from antiquity to the present day. It has been known since the 4^{th} century AD and it was widely used by the Greeks and Romans. It is of inorganic, mineral and synthetic origin. It is produced, initially, by oxidation of lead and then, by change to lead carbonate with vinegar and dung. Thin pieces of lead were placed with vinegar inside a sealed pot, which was placed inside dung for fifteen days. It becomes Yellow Massicot and then Red Litargirium by calcinations. It is soluble in citric acid.

It is resistant, very hard, with great opacity, density and brilliant whiteness. It can be mixed with all pigments, but with sulphur like cinnabar and cadmium, it changes and darkens. Byzantine painters usually used organic materials mixed

with lead white. It is used in oil paintings, tempera, encaustic, but not advised for fresco.

Chemical name: Basic lead (II)-carbonate

Crystal system: Trigonal

Refractive Index: 2.10

Grain size: Medium, 1-10 microns.

2.2.4.2.3 Red Lead

Red lead or Minium, Pb_3O_4 (Feller, 1985), is a pigment of inorganic origin. It is a pigment that has been used since antiquity, possibly since the discovery of lead. According to Pliny the Elder, the name Minium, was used for cinnabar, while red lead was called *Minium Secondarium*. This confusion was probably a result of the fact that cinnabar was often mixed with red lead. Gradually, however, the term "minium" came to be applied to red lead alone. Red lead is produced by heating white lead to 450° C.

It is an orange-red compound with very good covering power and good texture, but it is very reactive. It is soluble in acids, remains unaltered in alkalies, becomes brown in watery media, citric acid or acetic acid, white when exposed to hydrochloric acid and blackens to light exposure.

Chemical Name: Lead (II, IV) – oxide

Crystal System: Tetragonal

Refractive Index: 2.42

Grain Size: fine

2.2.4.3 Decomposition of the organic binding media

The prolonged exposure of the icons to unsuitable environmental conditions (sunlight, oxygen, humidity and air pollution), is responsible for the decomposition of the organic binding medium (Ioakimoglou, 1993) through *oxidative* and *hydrolytic* mechanisms.

However, apart from the environmental factors, the presence of other components, such as organic and inorganic pigments, lipids, hydrocarbons and others can have a destructive effect on the proteinaceous binders (Boon *et al.*, 1997).

Additionally, unsuitable conservation treatments and materials can be proved destructive.

Oxidative reactions

As long as the icon is exposed to the environment, the oxidative reactions will continue. This results to the weakening of the polyamide latex of the protein and the reduction of the binding ability.

Reactions of the proteins with lipids

In the mixed binding media, such as the emulsions, the oxidation of the protein is usually stronger. During the oxidation of the lipids, free radicals are being formed, which break the polypeptide latexes, thus creating free proteinaceous radicals and peroxides.

Reaction of the proteins with the additives

The painter, quite often, used several additives (vinegar, honey, gums, etc) to the binding medium in order to enhance its properties. In the case of egg tempera, the vinegar acts as an emulsifier and antiseptic. However, this acidic substance breaks down the binders via hydrolysis, leading to the reduction of their binding power.

Hydrolytic reactions

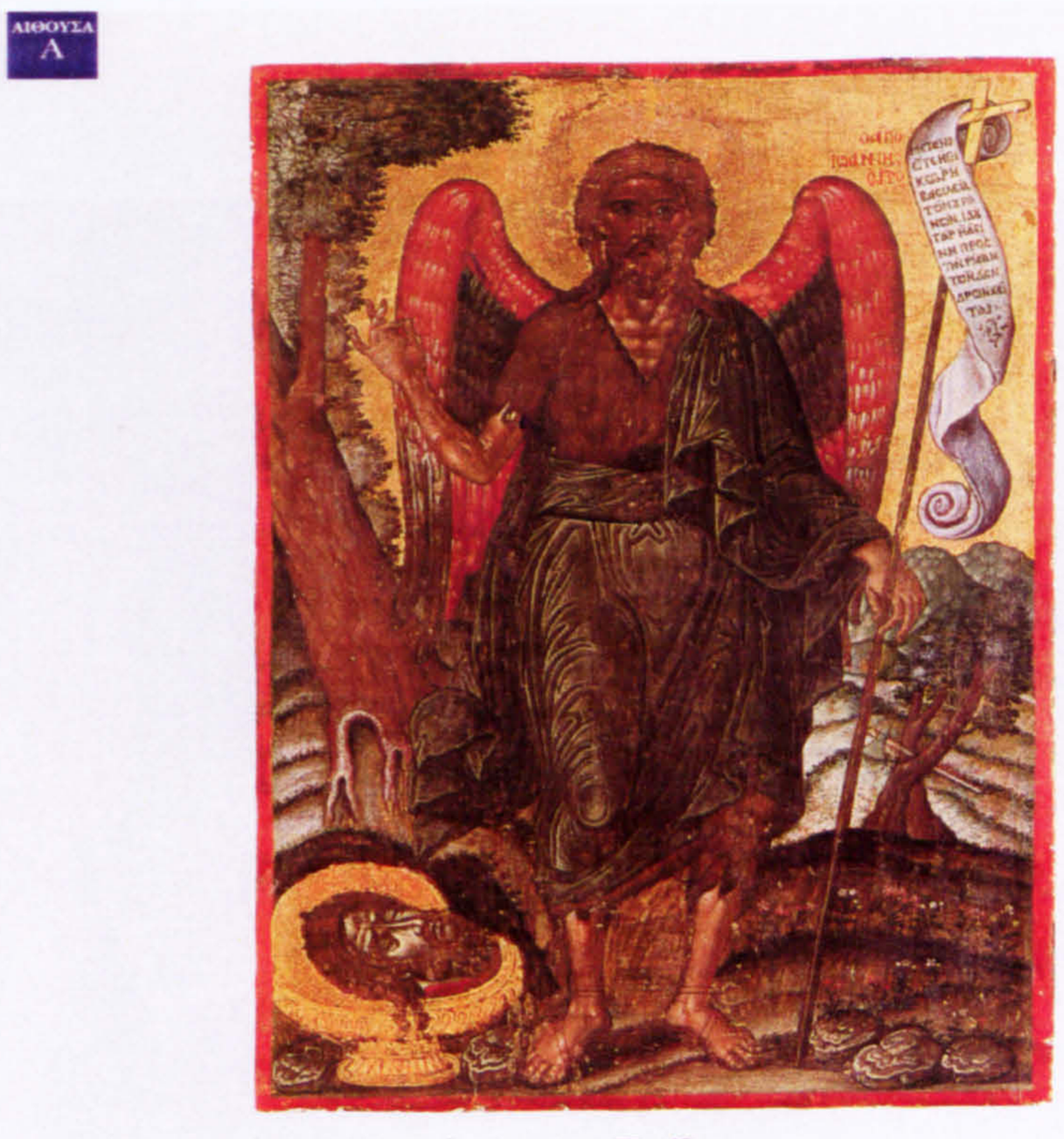
The high relative humidity favours the hydrolysis of the peptide bonds of the proteins, resulting in the formation of low molecular weight peptides.

CHAPTER 3

A REVIEW OF ANALYTICAL METHODS FOR THE CHARACTERISATION OF PIGMENTS AND BINDING MEDIA

The characterisation of the materials used by the artists was until recently a combination of hypothesis based on visual observation of the artefact and its' condition and historical sources describing the technique of the artist, if any. Characteristic of this model of identification is the catalogue of the Post-Byzantine Museum of Zakynthos (Mylona, 1998), in which the binding medium of the icons is described based purely on the conservator's "experience" (Figure 3.1).

Figure 3.1 Example of description of an icon from the catalogue of the Zakynthos Museum. The red circle shows the description of the technique: Oil-tempera (?) on wood with gesso ground



δ. Ο άγιος Ιωάννης ο Πρόδρομος

ΜΖ 39
Διαστ. 116×72×2 εκ.
Λαδοτέμπερα (;) σε ξύλο με προετοιμασία
Μέσα 18ου αι.

Ο Πρόδρομος εικονίζεται όρθιος, ολόσωμος, σε στάση αυστηρά μετωπική. Φοράει μηλωτή και ιμάτιο και φέρει φτερά. Υψώνει το δεξί χέρι σε ευλογία και με το αριστερό κρατάει ψηλή ράβδο, που απολήγει σε σταυρό, από τον οποίο κρέμεται ανοιχτό ειλητάριο με τη μεγαλογράμματη επιγραφή: *ΜΕΤΑΝΟ/ΕΙΤΕ ΗΓΓΙ/ΚΕ ΓΑΡ Η / ΒΑΣΙ-ΛΕΙΑ / ΤΩΝ ΟΥΡΑ/ΝΩΝ ΙΔΟΥ / ΓΑΡ Η ΑΞΙ/ΝΗ ΠΡΟΣ / ΤΗΝ ΡΙΖΑΝ / ΤΩΝ ΔΕΝΔΡΩΝ ΚΕΙ/ΤΑΙ* (Ματθ. γ' 2). Αριστερά κάτω είναι το κομμένο κεφάλι με φωτοστέφανο σε χρυσή λεκάνη με πόδι, που αναφέρεται στο μαρτυρικό τέλος και στην εορτή της Εύρεσης της Κεφαλής, και δεξιά στο δένδρο η αξίνα (Λουκ. γ' 9· Ματθ. γ' 10). Επάνω δεξιά με κόκκινο χρώμα στο χρυσό κάμπο η επιγραφή: *Ο ΑΓΙΟ(C) / ΙΩΑΝ-ΝΗC / Ο ΠΡΟ(ΔΡΟΜΟC).*

The use of science and technology for the determination of the chemical identity of those materials started in the second half of the 20th century (Hamsik, 1959; Lloyd Jones, 1962; Mills, 1966; Keck and Peters, 1969).

However, it was not until the 1990s that an increasing interest of the scientific community focused on the study of the artist's materials, offering a vast amount of knowledge and understanding along with a number of new investigative techniques.

This section outlines the analytical techniques used, from 1990 onwards, for the identification of the paintings' binding media and pigments.

3.1 Analytical techniques

3.1.1 Pigments

For pigment identification, a series of analytical methods have been used so far (Lahanier, 1991). Micro-chemical tests are invasive and destructive but can be (Cren-Olive *et al.*, 2000) useful for identification of organic dyes. Polarised light microscopy and scanning electron microscopy can be both invasive, sample size dependent, and non-destructive. They are based on the morphological characteristic of the samples. Infrared spectrometry has been used extensively for both organic and inorganic pigments, it is invasive, but it can be non-destructive. Burghio and Clark (2001) reported a non-destructive Raman investigation of pigments, the non-invasive nature of this investigation was indicated when testing was carried out *in situ*. Accordingly, Bikiaris et al. (1999) investigated the ochre differentiation of wall paintings in Greece through micro-Raman and micro-FTIR.

A range of X-ray techniques have also been reported (Aloupi *et al.*, 2000; Mantler *et al.*, 2000; Colombini, *et al.*, 2004). X-ray diffraction is an invasive and non-destructive method, quite popular for inorganic pigments. It measures the shape and the size of the crystal and this leads to the identification of the compound. Energy dispersive x-ray analysis (Aloupi *et al.*, 2000) is invasive but

non-destructive if the sample does not need to be gold-coated and gives elemental information of the inorganic compounds present in the sample. Finally, x-ray fluorescence, which is again invasive but non-destructive, has been used for the identification of pigments in a sample (Mantler and Schreiner, 2000).

3.1.2 Binders

The characterisation of organic binding media is not a simple process, since they have a quite complicated structure. The organic components undergo a chemical change during drying and ageing. The presence of some materials, such as several pigments, not only accelerates the ageing process, but can also contribute to the oxidation of the medium (Phoenix, 1997; Tumosa and Mecklenburg, 2004). This fact, together with the complexity of the various binding media, led to a series of analytical techniques for their identification and quantification.

Fluorescent staining techniques (Schaefer, 1997) have been widely used for the detection and identification of proteinaceous materials in samples taken from artefacts. The samples were exposed to staining reagents, responded according to the material present and the response detected under a fluorescent microscope. The same principle of microchemical tests, without using the materials' fluorescence was applied for the staining of cross-sections and the study of their response under a microscope (Cren- Olivé, 2000; Terlixi; 2006). These two techniques can indicate the class of medium present, i.e. protein or drying oil, but they cannot characterise the exact identity of the material.

Similarly, differential thermal analysis (DTA) has been employed for the detection of protein in the sample, but not for the characterisation of the exact type of the protein (Odlyha, 1995).

Immunological techniques, such as immunofluorescence (Kockaert *et al.* 1989) which is a method based on the reaction of the protein present to antiserum, produced by rabbits, have been investigated for the characterisation of proteinaceous binders. However, they require further optimisation in order to be adopted (Colombini and Modugno, 2004).

Furthermore, techniques such as direct pyrolysis mass spectrometry (DPMS), direct temperature resolved mass spectrometry (DTMS) (Colombini and Modugno, 2004) and pyrolysis-gas chromatography-mass spectrometry, (Py-GC-MS) (Scalarone et al., 2001; Bonaduce and Colombini, 2004) have been employed for the characterisation of the paint binding media. Also, capillary zone electrophoresis (CZE), matrix assisted laser desorption ionisation-mass spectrometry (MALDI-MS) and high performance liquid chromatography with electron spray ionisation-time of flight mass spectrometry (ESI-TOF MS) have also been tested for organic binding media identification but there is limited information on their application to samples from real artefacts (Boon *et al.*, 1997; Colombini and Modugno, 2004; Kuckova *et al.*, 2005).

Identification of proteins and oils can be possible by the non-destructive means of infrared spectroscopy (Derrick, 1994). However, in the case of organic mixtures, the interpretation of the spectra and the characterisation of the media present can be problematic (Colombini and Modugno, 2004). The determination is based on the detection of the amide linkages' bands characteristic of proteins and the detection of the triglycerides present in oils (Pilc and White, 1995; Kouloumpi, 2001; Kouloumpi *et al*, 2005). Meilunas *et al.* (1990) reported the characterisation of the aged binders using FTIR. A number of linseed oil, egg yolk and egg yolk/linseed oil emulsion reference samples were prepared by R. J. Gettens in the 1930s. In addition to these, Meilunas (1990) prepared and thermally aged another series of samples: linseed oil, lead white, stand oil, linseed oil with lead white, linseed oil with haematite, egg yolk, egg yolk with lead white and egg yolk with haematite. The FTIR spectra of those samples were obtained, in order to study and understand the process of ageing and

oxidation for comparison with the spectra taken from paint samples taken from two Italian Renaissance paintings. The first sample was from the "Principatus Angel" by Guarentio di Arpo and the second sample was from "Enthroned Madonna" by Antonio da Saliba. The results were quite successful. Identification of egg and oil was achieved, but it was quite difficult to detect egg yolk in the presence of oil (Meilunas, 1990).

In 2005, Van der Weerd *et al.* published a study of the influence of pigments on the ageing process of oily binding media, showing the importance and use of FTIR as an analytical technique leading to the characterisation of binders.

According to Vandenabeele *et al.* (2000, 2004) Raman spectroscopy can be of help for the identification of organic materials present in paintings. It is a non-destructive technique that has the ability to be applied *in situ,* which, like FT-IR, offers information on the chemical and structural composition of the sample for analysis. Thus, a series of proteinaceous, lipid resinous and polysaccharide media can be characterised. The identification of the proteinaceous materials is enabled through the detection of the amide functions for egg and the long-carboxyl chains in oils. Also, a quite extensive database of pigments and binding media has been created for identification purposes with Fourier Transform Raman spectroscopy (FT-Raman) (Burgio and Clark 2001). Additionally, Raman combined to Laser Induced Breakdown Spectroscopy (LIBS) has been used for the study of icons by Giakoumaki *et al.* (2006). Both spectroscopic methods (FTIR and Raman) are non-destructive, but they both present difficulties in identifying emulsions and the exact type of drying oils especially in mixtures.

The most widely used instrumental techniques which do permit the identification of the organic substances present in small paint samples even of complex mixtures, are the chromatographic techniques. Even though they are invasive and destructive, they have always been the preferred methods for binding

media identification, because the complex mixture of organic components can be separated and subsequently identified and quantified.

The early qualitative techniques of paper and thin-layer chromatography can only offer information regarding the general category of the binding medium. In general, they cannot differentiate between similar binding media such as egg and milk proteins (Vallance, 1997).

On the other hand, high performance liquid chromatography, HPLC (Halpine, 1992), atmospheric pressure chemical ionisation liquid chromatography (Shibayama *et al.* 1999), gas chromatography, GC (Husek, 1991; Nowik, 1995; Schilling and Khanjian, 1996; Schilling *et al.*, 1996; Ioakimoglou *et al.*, 2003), gas chromatography coupled to mass spectrometry, GC-MS (Casoli *et al.* 1998; Colombini *et al.* 1998; Colombini *et al.* 2002) have all been successfully employed for paint media characterisation.

Among all these chromatographic techniques, the most commonly used for the identification of proteinaceous and lipid binding media is gas chromatography alone, or coupled to a mass spectrometer. GC-MS allows the compounds from the analysis to be uniquely identified by a combination of retention time and mass spectral fragmentation pattern. In reality the molecular ion is used for identification, the fragmentation patterns of the esters are virtually identical.

In the gas chromatographic methods, the sample is hydrolysed mainly under acidic conditions (Vallance, 1997), to break down the material, to yield the aminoacids and fatty acids which are subsequently derivatized to render them amenable to chromatographic analysis.

The derivatization step is the most important step during the treatment of the samples and a variety of derivatization methods have been investigated. Among the most important ones (Vallance, 1997; Colombini and Modugno, 2004) are:

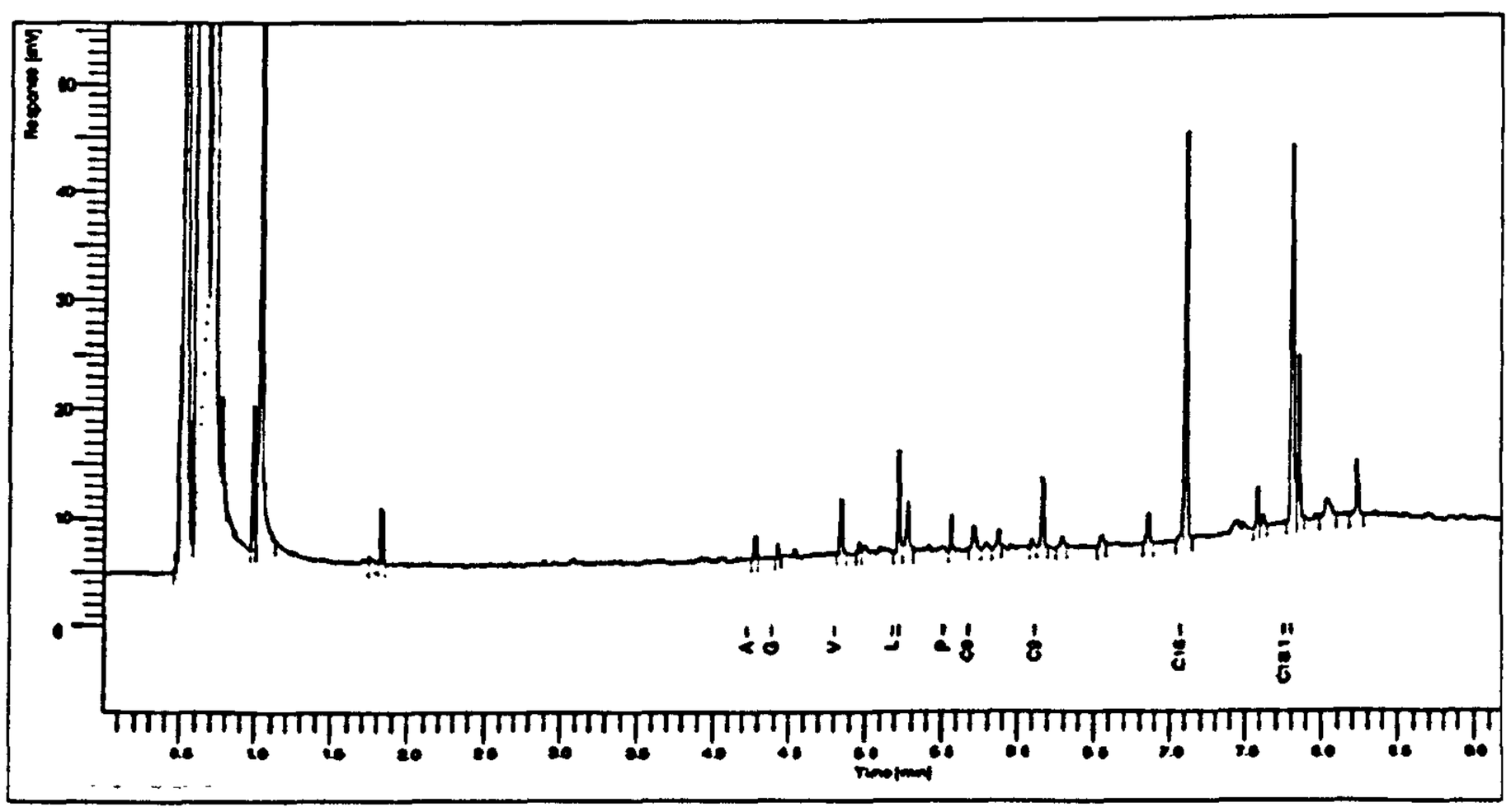
- Esterification and acylation, with the use of trifluoroacetic anhydride (TFA).

- Silylation with the use of *N,O*-bis(trimethylsilyl)acetamide (BSA), *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and N-tetra-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) derivatization agents.
- Derivatization with the use of ethyl chloroformate (ECF).

The first two methods were suitable for separate amino acid and fatty acid derivatization. The third method derivatized both fatty and amino acids simultaneously.

The interpretation and evaluation of the chromatographic analytical data is the step that leads to the identification of the organic binders. All chromatographic techniques require the creation of a database of known standards (Figure 3.2). There are several modes for binding media identification. The type of protein can be derived from the amino acid distribution (Phoenix, 1997; Ioakimoglou, 1993; Light and Smith, 1963), while the characterization can be based either on ratio flow charts, or on multivariate statistical analysis and correlation indexes (Colombini and Modugno, 2004).

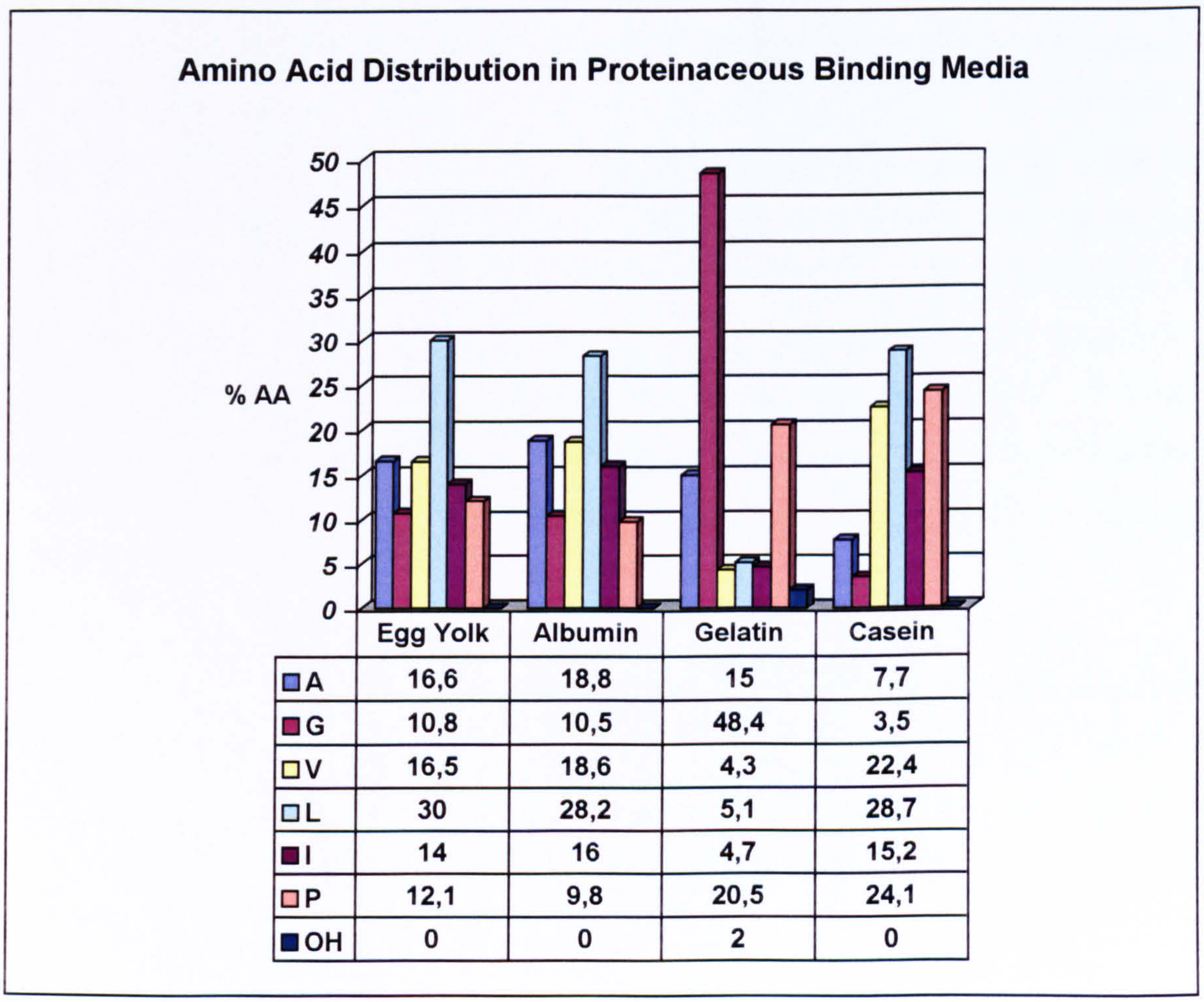
Figure 3.2 Typical GC trace of egg yolk reference sample



There have been several different approaches to the characterisation of proteinaceous media based on the detected amino acids ratios. Schilling et al.

(1996) for example considered the use of 14 amino acids but recognised that several of these were sensitive to acid hydrolysis conditions whilst tryptophane was completely eliminated. Schilling and Khanjian (1996) also showed that the seven amino acids: Alanine (A), Glycine (G), Valine (V), Leucine (L), Isoleucine (I), Proline (P) and Hydroxyproline (OH) were less affected by pigment interferences. Subsequent work by Terlixi et al (1998) and Ioakimoglou and co workers (1998) reported the use of the same seven amino acids for the identification of the proteinaceous binder materials in icons (Figure 3.3).

Figure 3.3 Chart of the relative amino acid distribution in the reference binding media



	Egg Yolk	Albumin	Gelatin	Casein
A	16,6	18,8	15	7,7
G	10,8	10,5	48,4	3,5
V	16,5	18,6	4,3	22,4
L	30	28,2	5,1	28,7
I	14	16	4,7	15,2
P	12,1	9,8	20,5	24,1
OH	0	0	2	0

The use of specific markers, such as hydroxyproline to detect the presence of animal glue and the use of the relative amino acid abundances are approaches

used by several authors, which in mixed systems may not be sufficient on their own for identification (Colombini and Modugno, 2004). An additional approach is the use of amino acid ratios. Several ratios have been proposed (Colombini *et al.*, 1998; Mateo-Castro *et al.*, 2001; Casoli *et al.*, 1998) such as Alanine/Proline and Alanine/Glycine. The amino acids chosen for the ratios may vary, but they are all usually based on the fact that specific amino acids are in excess in specific proteins, for example collagen-based glues contain high amount of glycine (Colombini and Modugno, 2004).

The use of fatty acid ratios has been adopted for the identification of lipid binding media and emulsions (Ioakimoglou and Yannovits-Argiriadi, 1993; Castro *et al.*, 1997; Casoli *et al.*, 1998). The most common fatty acids involved are: azelaic (C9), palmitic (C16), oleic (C18.1) and stearic (C18) with specific reference to the following ratios: C9/C16, C18:1/C18 and C16/C18 (Table 3.1). Mills and White (1994) have shown that the two saturated fatty acids C16 and C18 do not seem to alter significantly during ageing; therefore their peaks can be used for identification. The C9/C16 ratio can be used additionally for identification purposes, since the amount of C9 can be quite variable and it is hard to be certain about the reason for this (Mills and White, 1994). However, according to Schilling et al. (1996) azelaic acid, appears as a prominent peak in samples that contain drying oils and is much smaller in egg yolk and whole egg yolk samples.

On the contrary, the C18:1/C18 ratio cannot be used for identification purposes, but only as indicative of the ageing degree of the sample, due to the fact that oleic acid diminishes with ageing and it can be absent sometimes, indicating that the medium has undergone a long ageing process (Doménech-Carbó *et al.*, 2001).

Table 3.1 The fatty acid ratios of the reference samples

Sample	C9/C16	C18:1/C18	C16/C18
Poppy oil T° aged	0.4 ± 0.05	1.1 ± 0.1	3.9 ± 0.1
Walnut oil T° aged	0.7 ± 0.1	0.5 ± 0.05	3.3 ± 0.1
Linseed oil T° aged	1.0 ± 0.1	1.1 ± 0.1	1.6 ± 0.1
Egg yolk fresh	0.03 ± 0.02	2.8 ± 0.8	3.2 ± 0.3
Egg yolk aged T°	0.17 ± 0.02	2.2 ± 0.2	2.0 ± 0.1
Egg yolk aged T° + UV	0.07 ± 0.02	0.16 ± 0.2	2.4 ± 0.1
Egg tempera aged T°	0.05 ± 0.02	0.6 ± 0.2	2.5 ± 0.1
Egg yolk – Poppy oil T° aged	0.2 ± 0.05	0.4 ± 0.02	4.9 ± 1.4
Egg yolk – Walnut oil T° aged	0.3 ± 0.1	0.4 ± 0.1	2.9 ± 0.1
Egg – Linseed oil T° aged	0.5 ± 0.2	0.2 ± 0.05	1.8 ± 0.4

See Section 4.3.1 for the detailed ageing protocols

** T°- temperature*

3.2. Chromatographic determination of binding media from panel paintings

There are very few references to the analysis of icons from the Byzantine or Post-Byzantine eras and they mainly relate to the study of the paint layer structure and the characterisation of the pigments. An even more limited number of articles were found on the characterisation of Post-Byzantine binding media. On the contrary, quite a large number of articles have been written on the binding media identification of Western-European panel paintings.

Reviewing the literature published on the panel painting binding media identification, one notices that apart from isolated cases (Halpine, 1992; Van den Berg *et al.*, 2001), there are very few groups of scientists who focus on this field.

Ioakimoglou and Yannovits-Argiriadi (1993) studied the oxidative ageing mechanisms of egg yolk and linseed oil through gas chromatography-mass spectroscopy on artificial standards. They reported the use of hydrogenchloride methanol and 2,2 dimethoxypropane (DMP) for the methesterification of the fatty acids for the characterisation the binding medium of eleven Post-Byzantine icons belonging to the Benaki Museum, Greece and other private collections. In two of the icons, egg yolk was detected, while in the other nine the use of linseed oil was indicated, either in the form of an emulsion or as a contamination from subsequent layers.

In another GC-FID investigation by the same group (Ioakimoglou, *et al.*, 1998) on two Byzantine icons and a painting with a religious theme, the application of the ECF method, which was first introduced by Husek (1991), was reported. With the simultaneous derivatization of both amino and fatty acids, egg yolk was detected as the binding medium of the paint layers, either alone or mixed with animal glue, while the binder of the ground layers was animal glue alone and animal glue mixed with linseed oil. Another study with the same derivatization method of ECF (Terlixi, *et al.*, 1998) of samples taken from the painting "Evangelist Luca is painting Virgin Mary", detected the use of egg yolk as the paint's binding medium.

According to an article written by Casoli, A., *et al.*, (1998) four Post-Byzantine icons from the National Gallery-Alexandros Soutzos Museum of Athens, were analysed, in order to identify the binding media present. The aim of the experiment was to identify the presence of proteins and oils in the paint samples by GC/MS. Amino acid analysis verifies the presence of egg, but it does not distinguish egg yolk from egg white. Therefore, fatty acid analysis was also undertaken. Fatty acid analysis is also necessary for the detection of oils. The method employed was esterification and acylation, with the use of TFA. The results showed the presence of an emulsion of egg yolk and walnut oil for the paint layers, egg yolk for the layer of bole, and animal glue as the binder of the ground layers.

Another group, which has investigated the characterisation of binding media of icons with chromatographic techniques and mainly GC/MS is the Ormylia Diagnostic Centre. They have mainly worked on Byzantine and Post-Byzantine wall paintings but they have also carried out some analyses on Byzantine icons (Tsakalof *et al.*, 2001, 2003). For the determination of the binder, the analytical procedure they followed involved a complicated treatment of the sample starting with double extraction with chloroform in order to separate the proteinaceous sample from the other components, separate treatment of the organic and aqueous layers and derivatization with MTBSTFA. Their findings indicated the use of egg yolk as the main binder of the paint layers of Byzantine icons.

For the determination of binding media of Western-European panel paintings, there is a significant body of work – the salient features of which are discussed in the following section.

Halpine (1992) applied reversed phase – high performance liquid chromatography (HPLC-RP) for the characterisation of the paint binders of samples taken from Cosimo Tura's 15th century panel painting 'The Annunciation with St Francis and St Louis of Toulouse'. The results indicated that the artist used a variety of proteinaceous media in different coloured areas. For example, egg tempera for the red areas, animal glue for the blue-coloured areas and an egg/animal glue emulsion for the green zones.

Colombini's group has produced significant work on the chromatographic analysis of the organic materials, mainly from Italian wall paintings (Colombini *et al.* 1998; Colombini *et al.*, 1999a; Colombini *et al.*, 1999b), but also from a limited number of panel paintings (Colombini *et al.* 2002; Andreotti *et al.*, 2006). The methodology adopted by this group for the characterisation of the binding media present in both wall and panel paintings was similar to the procedure used by the Ormylia group. It involved double extraction, treatment of the organic and aqueous layers and derivatization with MTBSTFA and BSTFA reagents. Representative example is their analytical study on three paintings of

Cimabue (13th century), Raffaello (16th century) and Boucher (18th century). The GC-MS analysis indicated the presence of egg yolk, emulsion and linseed oil respectively (Colombini *et al.* 2002).

Schilling and co-workers (Schilling *et al.*, 1996; Schilling and Khanjian, 1996) investigated further the gas chromatographic method of ECF derivatives as established by Husek (1991) and developed by Nowik (1995). The advantage of minimum treatment of the samples and the simultaneous derivatization of both amino and fatty acids was applied and tested with positive results on samples from real artefacts (Schilling and Khanjian, 1996). The GC analysis of a ground sample removed from a 15th century panel painting indicated the presence of animal glue with oil as a contamination from the imprimatura layer. Furthermore, the analysis of the media present in some of Mantegna's paintings indicated the use of animal glue for the brown paints and egg yolk for the green paints. Likewise, samples taken from Brazilian altarpieces, suggested the presence of egg yolk and animal glue mixture.

Finally, there is another group that has focused on the GC-FID characterisation of artists' materials with ethyl chloroformate, especially from Spain and the area of Valencia (Gimeno-Adelantado *et al.*, 2002; Casas-Catalán *et al.*, 2004; De la Cruz- Cañizares *et al.*, 2004; Peris-Vicente *et al.*, 2006). Paint samples from an altarpiece painted by the Master of Alcira in Valencia, were analysed and the use of linseed oil and animal glue was confirmed. The binding medium of the panel painting "Virgen de los Desamparados" by Vicente López was found to be linseed oil (Mateo Castro *et al.*, 1997). In another analytical study of the paintings from the Basilica de la Virgen de los Desamparados (Doménech-Carbó *et al.*, 2001; Gimeno-Adelantado *et al.*, 2001 Gimeno-Adelantado *et al.*,2001) the GC-FID method of ECF derivatives indicated the use of different proteinaceous media in the ground samples (casein, animal glue) and the use of linseed oil in all paint samples.

This group has also investigated the use of HPLC for the characterisation of lipid binders from paintings with good results. The 2-nitrophenylhydrazides derivatives of fatty acids from five samples taken from five paintings again from Valencia, indicated the presence of walnut and linseed oil (Peris-Vicente *et al.*, 2004). Another analytical study of nine paintings with 4-bromomethyl-7-methoxycoumarin derivatization agent, showed the presence of linseed oil in all of the samples (Peris-Vicente *et al.*, 2005).

It can be seen from the section above that the available literature of the Byzantine and Post-Byzantine panel painting characterisation of binding media is extremely limited. Up-to-date, the number of icons investigated for characterisation of the binding media does not exceed fifty. The contribution of this thesis is therefore timely and significant.

CHAPTER 4

EXPERIMENTAL PROCEDURE

4.1 Developing a methodology

The characterization of the panel paintings' materials by analytical methods, contributes not only to the understanding of the artists' technique and the evolution of the painting styles but it also offers the conservator the background information needed for the proper treatment of the artefact and it gives a better understanding for the deterioration parameters.

Icons have been the subject of very little research, especially concerning the analysis of the binding media. Whilst the analysis of the inorganic components of a work of art has acquired a nearly routine character, the identification of the organic media still presents many difficulties, chief among these are the effects of ageing and the use of mixtures (Hugli, 1989, Masschelein-Kleiner, 1976; White, 1984; Phoenix, 1997). It is however from these analyses of the binders that the differences in the painting techniques used through the centuries can be understood.

However, the scientific analyses of the nature of these artefacts present severe difficulties. In particular, the determination of the organic materials from which the paint layers of a panel painting is made, is one of the most difficult problems for the analytical scientists. Similarly, a very important problem is the limited sample size, which can be usually less than 1mg and the small percentage of the organic binder which can be around 10%. Another important parameter is the inhomogeneity of the paint samples; pigments present, complicated composition of mixed proteinaceous media, as well as other materials present. All these can impede the analytical investigation of the constituency of a sample, or it can even give misleading results. The sample should therefore ideally be only the pigmented layer with all the varnish and the ground removed. This is not a trivial objective.

Therefore, the choice of proper analytical methods is very important and before designing the experimental procedure all those parameters have to be taken into consideration.

4.2 Sample Collection

201 samples were taken from 121 Post-Byzantine icons. The original idea was to take two to three indicative samples from each icon. The identification of pigments requires samples from all the pigments used, but in this case, identification of pigments was used to help the identification of the medium: non-destructive spectroscopic techniques, such as FTIR and Raman provide information concerning both organic and inorganic materials present in a sample. This fact can also hinder the understanding of a spectrum. Thus knowing the composition of an inorganic material such as a pigment present in a paint sample can facilitate the interpretation of the rest of the spectrum. Therefore no sampling from all the pigments present was considered necessary.

Mainly red and white coloured samples were collected, in order to optimise the methodology. Especially the choice of red was very selective, since there is a wide variety of red colours and it was based on a specific hue (orange-red), which is provided usually by cinnabar and red lead. The experience due to the conservation background of the author facilitated the collection of the red samples. As it has been explained in Chapter 2, cinnabar, red lead and lead white are typical inorganic pigments found in all three styles (Byzantine, Post-Byzantine and Western). SEM/EDX, on the other hand, detects and quantifies elements from which the pigment can be identified; therefore, a comparison on the constituency could easily be made.

At this point it is important to mention the quite complicated and time-consuming procedure with which a researcher is allowed to remove samples from artefacts in Greece. The researcher has to ask for permission through an application form, to the Ministry of Culture. Then after a few months, the applicant was

informed about the decision of the council (Appendix II). In cases where the objects are exhibited in a Church and they belong directly to the Cathedral and not the Ministry of Culture, then the applicant has to submit the form to the bishop in charge (Appendix II). In the course of this research there have been two applications of 50 icons in total, which have been rejected from the Ministry of Culture due to the rarity of the icons.

The samples from the icons were taken under a microscope, after the surface of the icon had been examined thoroughly using an optical microscope to identify previous conservation treatments and overpaintings. The samples were taken from areas, which were considered to be original and also, whenever possible, from areas that were damaged. The varnish was removed with a suitable solvent (acetone or ethanol), the paint layer was scraped off carefully with a scalpel and the powdered sample was placed into a sample vial. The paint layer was extremely thin and the collection of the powdered paint samples without removing part of the ground layer was very difficult. The weight of the sample varied between 0.5 to 1mg.

In some cases, the collection of powder paint samples was not possible either due to the extremely low thickness of the paint layers, or the bad condition of the object. In such cases, cross-sections from damaged areas of the icons were taken, in order to do micro-chemical tests and obtain schematic information about the type of materials used.

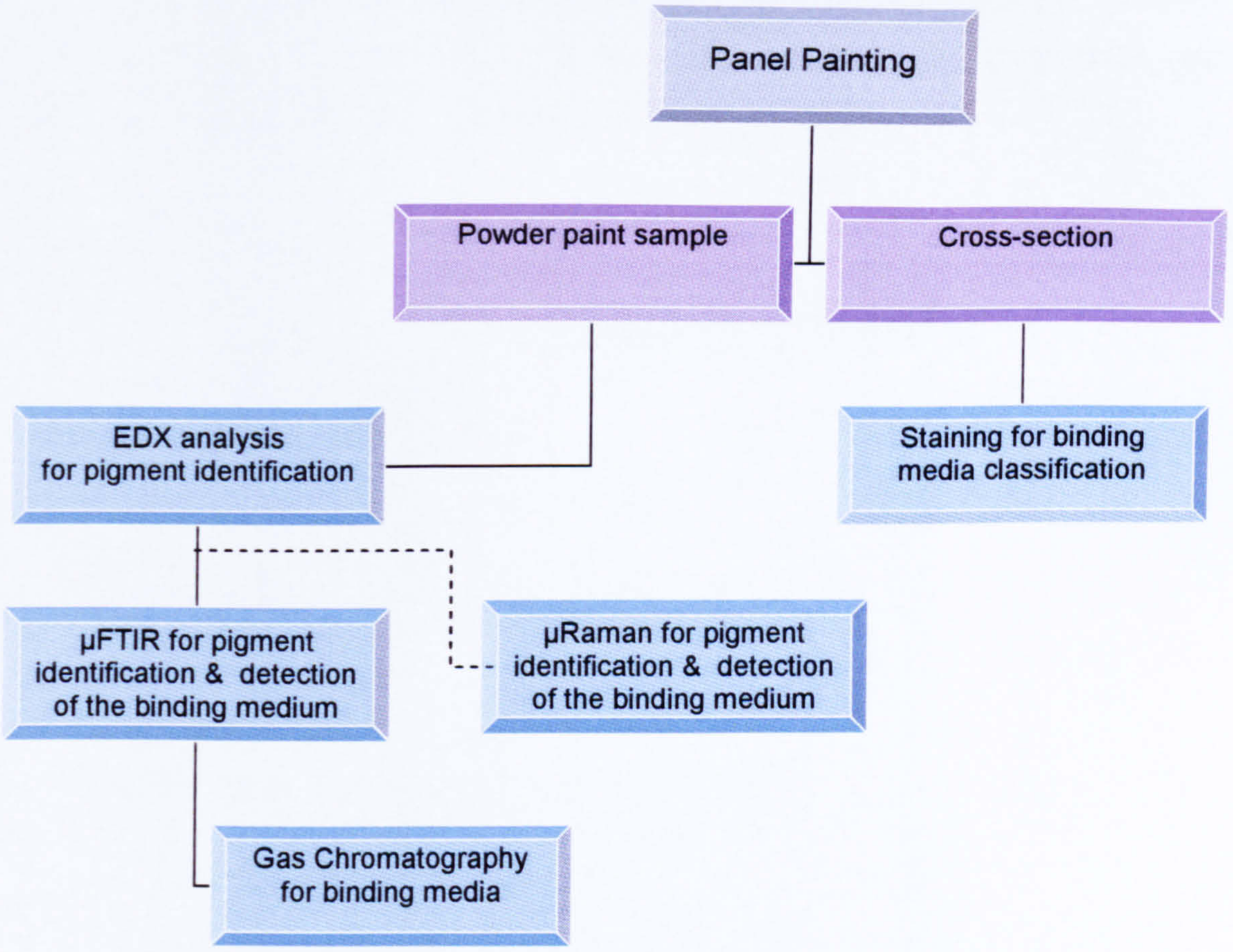
4.3 Analytical Scheme

Taking a sample from an artefact creates an ethical issue since it affects the integrity of the object; therefore both size and number of samples are very limited. This complicates things even more since most analytical techniques require a respectable amount of sample. Additionally, from the above-mentioned techniques the author could not have access to a series of them. Specifically, immunofluorescence and other fluorescent staining techniques could not be applied since De Montfort University does not have a UV

fluorescence microscope. Atmospheric Pressure Chemical Ionisation Liquid Chromatography also could not be applied, since it requires Electrospray, which, again, was not available. On the other hand, infrared spectroscopy (non-destructive) identifies all the components present and it cannot isolate the binding medium, unless there is a reference library installed in the hard disc. The chromatographic techniques (destructive) require hydrolysis of the protein and derivatisation of the amino acids and fatty acids, and the use of reference samples in order to identify the constituents.

Taking into consideration all the factors mentioned in the previous chapter, together with the fact that for any chromatographic technique the hydrolysis and derivatization of such small quantities of sample may result in the loss of the sample, it was decided to start the experiment with mainly non-destructive techniques, in order to obtain a first set of results and then proceed to a destructive technique, which appeared to be necessary for the positive identification of the organic binding medium. The analytical scheme chosen is the following (Appendix III):

Figure 4.1 Schematic representation of the experimental procedure



The analysis of the binding media is achieved either by the use of calibrated retention time data from standards or by the use of a GC-MS, which also allows structural information to be obtained from unknown or unexpected components. However, since GC-MS was not available at the Laboratory of Physicochemical Research of the National Gallery of Greece where most of the analytical part took place; the author was restricted to the use of calibrated GC-FID analyses.

4.3.1 Reference samples and database

Two series of reference samples were produced in 2001 by following the traditional recipes of both Byzantine (ek Fourna, 1906) and western techniques (Cennini, 1954). The one set did not undergo any cycling procedure, while the other set was thermally aged in order to understand and record the changes that occur with ageing, to compare it with the original paint samples and create a database for FT-IR and Gas Chromatography.

The reference samples (Table 4.1) for the binding media used were: egg yolk, three different types of linseed oil (raw linseed oil, stand oil and cold-pressed linseed oil), walnut oil, poppy oil, and emulsions of: egg yolk - linseed oil, egg yolk - walnut oil and egg yolk - poppy oil.

Table 4.1 The list of reference samples

No.	Reference Samples	Ageing
1	Egg yolk 1	
2	Egg yolk 2	
3	Raw Linseed oil	
4	Stand oil	
5	Cold-pressed linseed oil	
6	Walnut oil	
7	Poppy oil	
8	Egg yolk-linseed oil* 1:1	
9	Egg yolk-walnut oil 1:1	
10	Egg yolk-poppy oil 1:1	
11	Egg tempera	
12	White lead - egg tempera	
13	White lead - egg yolk -linseed oil 1:1	
14	White lead - egg yolk - walnut oil 1:1	
15	White lead - egg tempera	
16	Red lead - egg tempera	
17	Red lead - egg yolk - linseed oil 1:1	
18	Red lead - egg yolk - walnut oil 1:1	
19	Cinnabar - egg tempera	
20	Cinnabar - egg yolk - linseed oil 1:1	
21	Cinnabar - egg yolk - walnut oil 1:1	
22	Cinnabar - egg yolk - poppy oil 1:1	

Thermally aged at 120° C for 24h on 06/2001

**the term "linseed oil" is used to describe the use of raw linseed oil*

The choice of the drying oils was based on historical sources, which indicated that walnut oil was used in Italy during the 15th century; linseed oil in Northern Europe and adopted by the Italian masters during the 16th century. Poppy oil was used to a lesser extent, mainly in the 19th century French School. The inclusion of the three types of linseed oil in the database was considered necessary since artists' handbooks refer to their use (ek Fourna, 1906; Cennini, 1954). Gelatin, albumin and casein were included in order to compare the amino acid proportional difference with egg yolk. The pigments used were; cinnabar, red lead, and lead white. The choice of those three pigments was based on the fact that they were characteristic pigments of the artist's palette at that time (Feller, 1985).

For the preparation of the egg tempera samples, the egg yolk was separated from the egg white, and then removed from its skin. For one part of egg yolk, one part of vinegar and two parts of water were added. The mixture was then stirred very well and with the use of a brush, the pigment was mixed with the medium and then applied onto the glass microscope slides. For the samples containing egg and a drying oil, the process was similar to the one described above. The egg yolk was washed and removed from the skin. For one part of egg yolk, one part of oil and two parts of water were added. The emulsion had to be mixed by shaking very well, for the oil to break down. Then again with the aid of a brush, each pigment was mixed with the emulsion and applied onto a glass slide. The samples were left at room temperature for two days to dry before tested with FT-IR and then the one set was placed in the oven for the ageing procedure.

The accelerating ageing process followed was the oxidative type of ageing, based on the physical / chemical properties of the materials and on the theory that by increasing the temperature the rate of the reactions i.e. the materials degradation was increased (Feller, 1999). This scheme was also applied and tested by Meilunas *et al.*, (1990) and it is the one adopted in this research: "These films were identically treated in air at 120° C for 24 hours and cooled again to 25° C". The glass slides were placed in a Gallenhamp – Hotbox Oven of size 2, with stainless steel lining and fan for even heat distribution.

Additionally, another series of reference samples with a variety of ageing procedures (Table 4.2) obtained from the Conservation Department of the Athens Technological Educational Institute (TEI) was used for the enrichment of the database. Finally, it is important to mention the use of amino and fatty acid standards for the determination of the retention times of the target components (Table 4.3).

Table 4.2 Reference samples produced by Athens TEI

Reference Samples	Type/Code	Ageing
Egg yolk	TEI-standard board p4	Natural ageing 01/1998
Egg yolk- linseed oil* 1:1	TEI-standard board p4	Natural ageing 01/1998
Egg yolk - white lead 60%	TEI-standard board p4	Natural ageing 01/1998
Egg yolk - white lead 60% - linseed oil 1:1	TEI -standard board p4	Natural ageing 01/1998
Linseed oil-animal glue –chalk 1:1	TEI-koutsouris board (Γ_2)	Ageing under UV lamp (364nm) for 400hrs
Casein - Gesso ($CaSO_4 2H_2O$)	TEI-koutsouris board (Γ_4)	Ageing under UV lamp (364nm) for 400hrs
Egg yolk	Athens TEI	Temperature (120° C) and UV exposure (364nm) for 400hrs
Linseed oil	Athens TEI	Temperature (120° C) and UV exposure (364nm) for 400hrs

**the term "linseed oil" is used to describe the use of raw linseed oil*

Table 4.3 Standards used for the creation of the GC database

Amino acids	Fatty acids
1. Alanine	1. Azelaic acid
2. Arginine	2. Linoleic acid
3. Aspartic acid	3. Linolenic acid
4. Cysteine	4. Myristic acid
5. Glutamic acid	5. Oleic acid
6. Glycine	6. Palmitic acid
7. Histidine	7. Palmitoleic acid
8. Hydroxyproline	8. Pelargonic acid
9. Isoleucine	9. Ricinoleic acid
10. Leucine	10. Suberic acid
11. Lysine	11. Stearic acid
12. Methionine	
13. Phenylanine	
14. Proline	**Standard samples**
15. Serine	1. Casein
16. Threonine	2. Gelatin
17. Tryptophane	3. Albumin
18. Tyrosine	
19. Valine	
Amino Acid Standard	

4.3.2 Scanning Electron Microscopy / Energy Dispersive X-ray Analysis

X-rays techniques were used to identify the elements in the samples. This was achieved by measuring the energy of the x-rays emitted from a sample bombarded with either X-rays or a beam of electrons. The energies or wavelengths of the X-rays emitted by an element within a sample are characteristic of that element. In this investigation, samples were bombarded by an electron beam and the emitted X-rays were dispersed, detected and

measured. These experiments were carried out in a scanning electron microscope coupled to an EDX.

Scanning electron microscopy was first developed as an imaging tool for the topographical study of a sample. The sample to be analysed must be small enough to fit on the holder of the goniometer stage, which allows it not only to be moved horizontally and vertically but also to be rotated and tilted. The beam is focused on a fine spot on the surface of the specimen and the composition of that spot can be obtained. Low current and low voltage can make the study of conducting specimens possible without coating them. However, careless sample preparation can lead to faulty results by covering very fine details, important for the analysis of the sample.

Energy dispersive X-ray analysis is a very useful invasive and partially destructive technique for elemental analysis of inorganic materials. It can only detect elements with atomic mass number higher than 11. The spatial resolution is bigger than 25nm and the sampling depth is 500nm and is therefore surface sensitive. It is a powerful tool for the study of the paint layers and the identification of pigments. However, due to the irregularity of the paint sample and the heterogeneity of the surface, the technique is semi-quantitative.

4.3.2.1 Experimental

Each of the samples was mounted on aluminium stubs using a black double-sided tape, which contained a conductive adhesive. Since all the samples contained inorganic pigments, coating was not necessary.

4.3.2.2 Instrumentation

The instrument used for the analysis was a Leica S430 SEM with an Oxford ISIS 200 EDX. The acceleration voltage used was 20 kV and the probe current was 600 pico-Amperes. The instrument was calibrated before the analysis of the samples. Each sample was analysed twice, in two different areas of its

surface, to minimise the percentage of error. Image from the SEM, X-ray spectra and quantitative charts from the EDX were obtained for each sample.

4.3.3 Fourier Transform Infrared Spectroscopy

Infrared spectroscopy is one of the most important analytical examination methods in the field of Conservation. With this method classes of organic molecules (such as oils, resins, proteins, etc.) and inorganic compounds (minerals, pigments, etc.) and their mixtures can be identified.

The mid-infrared region of the electromagnetic spectrum extends over the range 4000-400 cm^{-1}. The energy of the radiation in this region is sufficiently high to excite molecular vibrational transitions within a sample.

Materials absorb different amounts of infrared radiation at different wavelengths depending on the chemical bonds that are present. With FTIR, chemical bonds and functional groups can be identified. Even similar compounds, such as isomers, can be distinguished because they have different spectra. Usually, it is wise to start to identify specific functional groups present in the compound. Scanning the sample in the region between 1500-3500 cm^{-1}, gives a spectrum that shows bands, corresponding with specific vibrations of functional groups and molecular bonds. By identifying the functional groups and their bonds, a compound can be classified (as an alcohol, phenol, etc.).

Scanning the sample in the region between 700-1500 cm^{-1}, gives the fingerprint of the sample, because in this region a unique set of absorption bands is being obtained.

The use of an IR microscope can solve problems of sample size and sample preparation. Only a few micrograms of sample are enough to produce a spectrum. The sample is placed in a diamond anvil cell and it is compressed between two diamond windows. IR microscopy consists of an optical microscope and an IR instrument with reflection optics to reduce the size of the beam, to that of the sample. This is an excellent method for infrared analysis of

art samples, since it is a non-destructive method and the sample can be used for other analyses.

Spectra showing the intensity of absorption at various wavelengths were interpreted to determine the type of bonds present and therefore, provide information about the composition. Precise identification was made by comparing the spectrum obtained with spectra of reference materials of known composition. Group correlation tables and charts giving wavenumbers or ranges of wavenumbers of all commonly encountered functional groups in organic molecules are widely available and provide a valuable aid to interpretation of spectra (Flet, 1963; Phillips, 1964; Nakamoto, 1986). In conservation science there are nearly 7000 spectra known and published. There are also electronic libraries with published data. One of the most important ones is the Irug Library (Infrared and Raman Users Group), which is continuously being updated, since research groups from all over the world submit their paint infrared spectra.

4.3.3.1 Experimental

All reference samples were run using an FTIR spectrometer and an FTIR spectrometer fitted with a microscope, both provided by De Montfort University. The purpose of this was to ensure that high quality spectra were obtained and the possibility of error was reduced. The original paint samples were run only on the FTIR microscope due to the nil sample preparation.

The samples that were applied onto KBr discs, were placed into the KBr disc holder, inserted into the sample holder, and their spectra were recorded. The samples that were applied onto glass slides were prepared as KBr discs. A small quantity of the sample was scraped from the surface and ground with spectrograde potassium bromide (KBr) in an agate pestle and mortar. The powder was pressed in a die under 10 tones of pressure for 2-3 minutes. The disc was placed in the KBr holder, then in the sample cell and its spectrum was recorded. It is important to note that before recording the spectrum of each

sample, the background was recorded so that it could be subtracted subsequently.

The KBr discs were also recorded using the Nicolet spectrometer. The microscope was not used for that process. No sample preparation was necessary, since the discs were ready. The samples were placed in the KBr holder and their spectra were recorded.

Each sample was recorded at least twice and whenever the spectrum was not satisfactory, a new KBr disc was produced. The computer did not have a library of spectra useful for this experiment; therefore the comparison of the spectra produced against any database proved difficult. In addition, the subtraction of spectra generated poor results. However, the results were interpreted with the aid of published articles and published libraries of spectra.

The preparation for the samples to be run under an FTIR microscope involved the following: The samples were placed under a stereomicroscope and with the aid of fine tungsten needles; a minute quantity was removed and placed in a compression cell between diamond windows. The sample was, then, placed under the microscope, the background was run and then the sample was run to record its spectrum.

4.3.3.2 Instrumentation

A SHIMADZU HYPER IR for the FTIR 8000PC Series Spectrometer was used connected to a Dell computer. The analysis was carried out in the transmission mode. The spectrum was recorded after 10 scans, with a resolution of 4 cm^{-1}. The infrared spectra obtained were from the mid-infrared region of 3998.16 to 401.17 cm^{-1}.

The second instrument to be used was a Nicolet 5DXC FTIR Spectrometer coupled to a SpectraTech IR-PLAN^{TM} Infrared Microscope Accessory and a MTC (Mercury Cadmium Telluride) detector cooled with liquid nitrogen. The

analysis was carried out in the transmittance mode. The spectra were recorded after 128 scans, with a resolution of 8 cm^{-1}. The spectra obtained were from the mid-infrared region of 4000 to 650 cm^{-1}. The aperture below and above the sample was 100 microns wide and 50 microns long.

A third instrument was also employed for the original paint samples: a Perkin Elmer Autoimage FTIR microscope system and a MTC detector cooled with liquid nitrogen. The analysis was carried out in the transmittance mode. The spectra were recorded after 128 scans, with a resolution of 8 cm^{-1}. The spectra obtained were from the mid-infrared region of 4000 to 700 cm^{-1}. The aperture below and above the sample was 100 microns wide to 50 microns long.

4.3.4 Raman Spectroscopy

Raman spectroscopy was not used as part of the analytical protocol established by this research. It was used for a set of original paint samples, in order to compare the amount of information obtained with μFT-IR.

The theory behind Raman spectroscopy is similar to the one for infrared spectroscopy (Lewis and Edwards, 2001). Each band of the Raman spectrum corresponds to a particular molecular vibration within the material. Therefore in a Raman spectrum of a material a pattern of bands is typical of "groups" of atoms which can be identified in different samples as the "finger print" which identify these groups in different molecules and structures. The technique involves shining a monochromatic light source on a sample and detecting the scattered light. The majority of the scattered light is of the same frequency as the excitation source; this is known as Rayleigh or elastic scattering. A very small amount of the scattered light is shifted in energy from the laser frequency due to interactions between the incident electromagnetic waves and the vibrational energy levels of the molecules in the sample. Plotting the intensity of this "shifted" light versus the energy difference (expressed as wavenumbers) results in a Raman spectrum of the sample. Generally, Raman spectra are plotted with respect to the laser frequency such that the Rayleigh band lies at 0 cm^{-1}. On this scale, the band positions will lie at frequencies that correspond to

the energy levels of different functional group vibrations. The Raman spectrum can thus be interpreted similarly to the infrared spectrum.

However, whereas IR bands arise from a change in the dipole moment of a molecule, Raman bands arise from a change in the polarizability. In many cases, transitions that are allowed in Raman spectroscopy are forbidden in IR, so these techniques are often complementary. By using μRaman spectroscopy, Raman spectra of microscopic regions of samples can be measured.

4.3.4.1 Experimental

No sample preparation was required for Raman analysis under the Raman microscope.

4.3.4.2 Instrumentation

A Renishaw System-1000 spectrometer (Wotton-Under-Edge, UK) with a diode laser with a laser wavelength of 785 nm and an output power of 50 mW was employed. Laser intensity on the sample could be modified up to ca. 5 mW, by using a set of neutral density filters. In order to take possible sample inhomogeneity into account, for each selected sample at least 3 Raman spectra were recorded by using the 50X objective lens, allowing for a spectral footprint of ca. 2 μm. All spectra were recorded between 200 and 1800 cm^{-1} for 5 accumulations of 30 s.

4.3.5 Gas Chromatography

The gas chromatographic analysis of the fatty acids and amino acids content in binding media has been used to identify egg tempera and emulsions of proteins with drying oils (Mills and White, 1994). However, the organic binders are compounds of high molecular weight, which need to be suitably treated before their introduction to the Gas Chromatographer for analysis. This fact leads to two important steps:

a) The reduction of the molecular weight of the natural polymers through acid hydrolysis, and

b) The reduction of the polarity through conversion of those components to less polar derivatives, where the hydrogens have been substituted from other groups: R-, RCO-, $(CH_3)_3Si$- etc, i.e. the alcohols into ethers, the amino acids into amides and the carboxylic acids into esters.

As mentioned in Chapter 3, there are several methods for the treatment of the samples before their introduction in the GC column.

The formation of ethyl chloroformate derivatives via derivatization with ethyl chloroformate was the method adopted by this research. The choice was based on the fact that it provided the capability for simultaneous determination of both amino acids and fatty acids. This method has been reported by Husek, (1991), Nowik, (1995), Schilling and Khanjian, (1996) and Schilling *et al.* (1996), Mateo Castro *et al.* (1997) and has been shown to be quick and capable of producing results from a few mg/sample.

4.3.5.1 Experimental

The analytical procedure consisted of three main steps: acid hydrolysis, derivatization (ECF derivatives of amino acids and fatty acids) and chromatographic analysis.

Hydrolysis

The samples were placed in Pyrex tubes with screwed Teflon caps, in which 150 μl of 6N HCl were added. The hydrolysis lasted for 24 h at 110^0C. During the hydrolysis proteins were broken down into their constituent amino acids and lipids into their fatty acids. After hydrolysis the mixture was neutralized with $CaCO_3$ (0.035g).

Derivatization

50μl of the hydrolyzed sample were treated with 50 μl of ethanol/pyridine (4:1v/v) and 15 μl of ECF were added by briefly stirring the vial. At the end of the reaction, which lasted about 20 sec, 50 μl of ethyl acetate were added to the

reaction mixture to extract the derivatives into an organic phase. 1µl of the organic phase was injected into the GC column.

4.3.5.1.1 Media Identification

As previously reported (Chapter 3) there have been several approaches to the characterisation of organic binding media. In this research, the characterisation of the organic binders was based on a combination of methods. For the proteinaceous binders the relative abundance of the amino acids along with the use of amino acid (AA) ratios was employed. The use of fatty acid (FA) ratios was applied for the determination of the lipid and mixed binders. The use of all three methods were employed for the detection of egg yolk in the samples

4.3.5.1.2 Materials

Chemicals and Reagents: Ethyl chloroformate (ECF) was purchased from Merck (Darmstadt, Germany). Pyridine and ethyl acetate were obtained from Fluka (Buchs, Switzerland)

Reference materials: The amino acid standard used was from Sigma (St. Louis, MO, USA) Palmitic acid, stearic acid, oleic acid and azelaic acid, gelatine, albumin and casein were obtained from Fluka. Egg yolk was obtained from the market. Linseed oil, walnut oil, cinnabar, red lead, and white lead were obtained from Fitzpatrick (London, UK).

4.3.5.2 Instrumentation

A model 8700 Perkin Elmer gas chromatograph with a flame ionization detector was employed (Rood, 2007). Helium (99.999%) was used as the carrier gas. The chromatographic separations were achieved on a 15m x 0.25mm ID column from Restek (RTX-1701), using the following temperature program: The initial temperature was 70°C for 1 min, and then it was increased at 27°C /min up to 250°C, where it was maintained for 10 min. The injector and detector temperatures were 240°C and 260°C, respectively. The helium head pressure

was 17 psig. The split ratio was 20:1. The relative standard deviation (RSD) was around 5% on 5 subsequent runs for each sample.

4.3.6. Optical microscopy & microchemical tests

By this method (Johnson and Packard, 1971; Terlixi, 2006), cross-sections of micro-samples removed from paintings can be studied under an optical-metallographic microscope. Thus, information concerning the stratigraphy of the artefact, as well as the thickness and the nature of the layers can be obtained.

Apart from the visual observation of the samples under the microscope, a series of micro-chemical tests have been developed in order to characterize, at a first level, the class of the organic media present.

A range of organic dyes have been identified (Cren-Olive *et al.* 2000) that will bind to different proteinaceous material and indicate the presence of selected species (Table 4.4). The different reactions were determined by the differences on the amino acid groups and thus are reagents that detect amino groups, phenyl groups, carboxyl groups, etc.

Table 4.4 Important dyes for the indication of proteinaceous materials.

REAGENT	STAIN	DETECTION
Fuschine S	Red	Proteins
Noir Amide	Blue	Proteins
Oil Red	Red	Lipids

The samples were embedded in polyester resin, polished and burnished, and then studied under the optical microscope.

Then, for the staining of the cross-sections a few drops of the colorant were dripped on the surface of the sample and after around 10 minutes, they were rinsed off. If the sample had been stained, then the type of the binder was characterized (protein or lipid). Even though this method can give information

about the class of the binder, it cannot identify the exact type of the medium. For example, the presence of a protein can be detected, without offering any more information about the exact type of the protein.

4.3.6.1 Experimental

For the current research, Noir Amide 2 (pH:2) was used: 10mg of Naphtol Blue Black was dissolved in 4.5ml of acetic acid, 4.5ml of sodium acetate (0.1mol/L) and 1ml of glycerol. This colorant has the ability to combine with proteins and has a strong reaction to egg (Cren-Olive *et al.* 2000). In case the sample contains a proteinaceous medium, it will stain, otherwise, if it is of a lipid origin, it will not.

4.3.6.1.1 Materials

Naphtol Blue Black, acetic acid, sodium acetate and glycerol were obtained from Fluka (Buchs, Switzerland).

4.3.6.1.2 Instrumentation

A Leica DLML optical metallographic microscope was employed. The software used for the study of the samples was the IM50.

4.4. Analysis of Reference Samples

Reference samples of binding materials were analyzed in order to provide information to facilitate the identification of the binding media used in panel paintings.

4.4.1. SEM/EDX

EDX analysis of the pigments before the preparation of the reference samples was considered necessary in order to ensure that materials of known composition would comprise the database.

Having elemental information of the pigments present would also help with the interpretation of the infrared spectra. EDX analysis of the three pigment

samples gave quantitative elemental information on their constituents. This quantitative data may not be representative of a sample which may be a mixture of pigments in the area sampled. However, two different areas of the same sample were tested and the results were the same. EDX analysis of lead white, red lead and cinnabar (Table 4.5) showed that all three pigments were pure with no additives.

Table 4.5 Results of EDX analysis of the selected pigments

Samples	Elements			
	Pb	O	Hg	S
Lead white	87.75%	12.25%		
Red lead	91.69%	8.31%		
Cinnabar			95%	5%

4.4.2 FTIR

As previously mentioned, FTIR is a non-destructive method which can provide the researcher with valuable information concerning the composition of a material. Also it offers information on the organic material present but the interpretation of a spectrum can be quite complicated especially when mixtures of materials are involved. Thus, the formation of an adequate database that would provide reference on the absorption bands of the materials of interest along with an understanding of the ageing mechanisms was of primary importance.

Drying oils: The absorption peaks of both fresh and aged samples are the ones expected and very well defined. It is clearly seen in Table 4.6 that the results are almost identical for all three oils. In the spectra of the aged drying oils, the following are noticed: the peak at the 3467.8 cm^{-1} is possibly due to the formation of oxidation products, exhibiting hydroxyl. The absorption band at 3010.7 cm^{-1} disappears with ageing. The intensity of the bands attributed to CH_2 stretch reduces with ageing and this is probably due to the loss of some hydrocarbons.

Table 4.6 Drying oils

Interpretation	Linseed oil		Poppy oil		Walnut oil	
	Fresh	Aged	Fresh	Aged	Fresh	Aged
Oxidation products	-	3467.8 m	-	3467.8 m	-	3467.8 m
Olefinic stretching band [(=C-H)stretch]	3010.7 m	-	3008.7 m	-	3008.7 m	-
CH_2 stretch	2958 sh	2958 w.sh	2958 sh	2958 w.sh	2958 sh	2958 w.sh
CH_2 stretch	2925.8 s	2927.7 m	2925.8 s	2927.7 m	2925.8 s	2927.7 m
CH_2 stretch	2854.5 s	2854.5 m	2854.5 s	2854.5 m	2854.5 s	2854.5 m
Carbonyl band [C=O stretch]	1745.5 s	1739.7 m	1745.5 s	1741.6 s	1745.5 s	1741.6 m
(-HC=CH-) stretch	1652.9	1635 w. sh	1652.8	1635.5 w. sh	1652.8 w	1635 w. sh
C=C aromatic and conjugated	1463.9 m	1461.9 m	1463.9 m	1458.1 m	1463.9 m	1463.9 m
C-O stretch	1417.6 w	1417.6 w	1420 w	1417.6 w	1417.6 w	1417.6 w
	1375.2 w	1377.1 w	1377.7 w	1375.2 w	1377.1 w	1377.1 w
Triglyceride ester linkages	1242 m	1240.1 m	1242 m	1240.1 m	1245.9 m	1240.1 m
Triglyceride ester linkages	1163 s	1166.9 s	1163 s	1166.9 s	1163 s	1168.8 s
Triglyceride ester linkages	1099.3 m	1099.3 m	1099.3 m	1099.3 m	1099.3 m	1099.3 m
Aromatic C-H out-of-plane	970 w	975.9 w	970 w	975.9 w	970 w	975.9 w
CH out-of-plane [(=C-H)$_\delta$]	721.3 w	725.2 w	723.3 w	725.2 w	721.3 w	725.2 w
Hydrogen bonded, out-of-plane O-H bond	669.3 w	667.3 w	669.3 w	667.3 w	669.3 w	-

All data are in wavenumbers (cm^{-1})

Egg yolk: Proteins, which are unique to egg yolk, are built up from amino acids. It is therefore evident that they are characterised by amide bonds and those characteristic of egg yolk are shown in Table 4.7. The absorption peaks present in the spectrum of fresh egg yolk are present in the spectrum of the aged egg. A slight shift is noticed in some peaks probably as a result of the ageing process. It has also been noticed that the intensity of some of the absorptions has been reduced with ageing. The intensities of the absorption bands of the triglycerides ester linkages have noticeably been reduced. The results obtained are in agreement with the work done by Meilunas (1990).

Egg tempera: As it can be seen in Table 4.7 in the results obtained for egg tempera the absorption bands are very close to the ones for the egg. Some shifting has been noticed and this was probably due to the addition of water and vinegar. The aged egg presented a reduction to the intensities of some absorption bands, which may be due to the evaporation of the water and the formation of oxidative products.

Table 4.7 Egg

All data are in wavenumbers (cm^{-1})

Interpretation	Egg yolk		Egg Tempera	
	Fresh	Aged	Fresh	Aged
Oxidation products	-	3421.5 m	3436 w	3421.5 w
N-H stretch	3288.4 m	3290 sh	3290 w	3290 w
Amide II overtone	3080 w	3080 w	3080 w	3080 w
Lower concentration of unsaturated chains	3006 w	3006 w	3006 w	3006 w
CH_2 absorption band	2923.9 s	2923.9 m	2926.7 s	2925.8 s
CH_2 absorption band	2852.2 s	2852.5 m	2854.5 s	2854.5 m
Triglyceride ester linkage	1745.5 sh	1749.3 m	1742.6 s	1749.3 m
Amide I [C=O stretch]	1656 s	1656 m	1660.8 s	1654.8 m
Amide II [$(NH_2)_\delta$]	1631.7 s	1633.6 m	1632 m	1637.5 m
Amide II	1546 m	1548 w	1548 m	1542.9 m
	1465 s	1456 w	1458.6 m	1458.1 w
C=C aromatic and conjugated	-	1456 w	-	-
C-N stretch of primary amides	1379 w	1379 w	1386.4 w	1361.7 w
Triglyceride ester linkages	1236.3 w	1236 w	1242 w	1245 w
Triglyceride ester linkages	1164.9 s	1168.8 w	1160.2 m	1170.7 w
Triglyceride ester linkages	1093.6 m	1093.6 w	1088 w	1091.6 w
Aromatic C-H out-of-plane	970.1 w	970 w	972.5 w	968.2 w
Lower concentration of unsaturated side chains	721.3 m	721 w	717.4 w	720 w
Hydrogen-bonded, out-of-plane O-H bend	669.3 w	669.3 m	664.4 w	669.3 s

Emulsions: As it can be observed from Table 4.8, the absorption bands of emulsions comprising from linseed, poppy or walnut oil mixed with egg yolk are almost identical. The triglyceride units composed of unsaturated fatty esters are common to both egg yolk and the drying oil present. However, the proteins in the egg yolk provide absorption peaks, which are characteristic for the chromophoric amide linkages. These are extremely durable and they are present even when the egg is being mixed with oil. The aged egg/oil emulsions present a slight shifting of the absorption peaks identified in the fresh samples and this may be due to the evaporation of water and the formation of oxidation products with ageing.

Table 4.8 Emulsions

Interpretation	Egg/Linseed oil		Egg/Poppy oil		Egg/Walnut oil	
	Fresh	Aged	Fresh	Aged	Fresh	Aged
Oxidation products	3451.3 m	-	3454.3 m	-	3444.6 m	-
N-H stretch	3302.1 m	3296.1 m	-	3294 m	3312.6 m	3296.1 m
Amide II overtone	3080 w	3080 w	-	3080 w	-	3080 w
Olefinic stretching band [(=C-H)stretch]			3012.6 w	-		
Lower concentration of unsaturated chains	3006 w	3006 w	-	3006 w	-	3006 w
CH_2 stretch	2926.7 s	2960 sh	2925.8s	2925.8 sh	2925.8 s	2925.8 sh
CH_2 stretch	2854.5 s	2854.5 m	2854.5 s	2854.5 m	2854.4 s	2854.5 m
Thiglyceride ester linkage	1742.6 s	1743.5 s	1745.5 m	1743.5 s	1745.5 s	1741.6 s
Amide I [C=Ostretch]	1660.8 m	1660 w	1651 m	1660 w	1660 m	1660 w
(-HC=CH-)stretch	1645 wsh	1633.6 w	1645 wsh	1633.6 w	1635.5 wsh	1637.5 w
Amide II	1540.4 m	1537.1 w	1541 m	1537.2 w	1540 m	1541 w
C=C aromatic and conjugated	1468 m	1460 w	1465 m	1460 w	1463.7 m	1460 w
C-O stretch	1415 w	1417.6 w	1415 w	1417 w	1415 w	1417 w
C-N stretch of primary amides	1381.6 w	1375.1 w	1377.1 w	1377.1 w	1387 w	1375.2 w
	1340 w	1360 w	1340 w	1360 w	1340 w	1340 w
Triglyceride ester linkages	1246.8 w	1234.6 w	1238.2 w	1235 w	1246 w	1234.3 w
Triglyceride ester linkages	1165 m	1168.8 m	1164.9 m	1168.8 m	1166.8 m	1166.9 m
Triglyceride ester linkages	1102.4 w	1095.5 w	1091 w	1097.4 w	1099 w	1099.3 w
Aromatic C-H out-of-plane	977.3 w	970.1 w	970 w	975.9 w	977 w	970.1 w
	760.7	-	760.7	-		
Lower concentration of unsaturated side chains	727 w	720 w	720 w	723.3 w	727 w	723.3 w
Hydrogen-bonded, out-of-plane O-H bend	669.25 w	667.3 m	667.3 w	667.3 w	669.3 w	669.3 m

All data are in wavenumbers (cm^{-1})

Cinnabar and binders: As it can be seen (Table 4.9) from both spectra of fresh and aged cinnabar-egg tempera, the absorption peaks are nearly the same as the ones for the egg tempera. Cinnabar has no significant absorption in the mid-infrared region and it does not affect the spectrum. The absorption band at 3427.3cm^{-1} for the aged sample does not exist in the spectrum of the fresh sample. This may be due to water absorbance of the KBr disc. Similarly, the absorption band at 3288.4cm^{-1} for the N-H stretch at the fresh sample, seems to be masked in the aged sample from the 3427.3cm^{-1} absorption band.

Similarly, the bands for both fresh and aged cinnabar-egg yolk-linseed oil were quite close to the ones without the cinnabar (Table 4.9). The bands, which characterise the egg were present but not intense. The band at 3286.5cm^{-1} for the N-H stretch seems to have been masked from the absorption band of 3435cm^{-1} at the sample of the aged egg. The band at 669.9cm^{-1} for the sample of the aged egg has vanished on the sample of the fresh egg. The results show that it is quite difficult to characterise egg yolk in the presence of linseed oil, especially for the aged sample, where the intensities of the absorbance have been reduced.

Table 4.9 Cinnabar and binders

All data are in wavenumbers (cm^{-1})

Interpretation	Cinnabar/egg tempera Fresh	Cinnabar/egg tempera Aged	Cinnabar/egg/ linseed oil Fresh	Cinnabar/egg/ linseed oil Aged
Oxidation products			-	3435 s
O-H stretch due to water absorption	-	3427.3 m		
N-H stretch	3288.4 m	-	3286.5 w	-
Amide II overtone	3080 w	3080 w	3080 w	3080vw
Olefinic stretching band [(=C-H)stretch]			3008.7 w	3008.7 vw
Lower concentration of unsaturated chains	3006 w	3006 w		
CH_2 band	2925.8 s	2925.8 s	2925.8 s	2925.8 s
CH_2 band	2854.5 s	2854.5 m	2854.5 m	2854.5 m
Trygliceride ester linkage	1743.5 s	1743.5 s	1745.5 m	1741.6 m
Amide I [C=O stretch]	1651 s	1652.9 m		
(-HC=CH-)stretch			1645.2 w	1637.5 w
Amide II	1546.8 m	1542.9 w	1542.9 w	1542.9 w
C=C aromatic and conjugated	1460 m	1458.1 m	1458.1 w	1458.1 w
C-N stretch of primary amides	1384.8 w	1379 w	1375 w	1375 w
Triglyceride ester linkage	1238.2 w	1236 w	1236.6 w	1236 sh
Triglyceride ester linkage	1161.1 m	1163 m	1163 w	1163 w
Triglyceride ester linkage	1093.6 w	1093.6 m	1093.6 w	1095.5 w
Aromatic C-H out-of-plane	975.9 w	970 w	974 vw	970 vw
Lower concentration of unsaturated chains	720 w	720 w	721.3 w	720 vw
Hydrogen-bonded, out-of-plane O-H bend	671.2 w	652 w	-	669.3 w

Lead white and binders: As it can be observed from the absorption bands of both fresh and aged lead white-egg tempera sample (Table 4.10), the pigment has slightly affected the spectrum. Due to its strong peaks, some of the bands belonging to the egg have been reduced in intensity, e.g. the triglyceride ester linkages.

Moreover, the spectra of both fresh and aged sample seem very similar. White lead affects quite strongly the spectrum of egg yolk-linseed oil. The N-H stretch at 3080cm^{-1} disappears in the aged sample. The anti-symmetric CO_3 stretching vibration at 1396.4cm^{-1} seems to masks the absorption bands of the triglyceride ester linkages resulting in very weak peaks.

Table 4.10 Lead white and binders

All data are in wavenumbers (cm^{-1})

Interpretation	Lead white/ egg tempera Fresh	Lead white/ egg tempera Aged	Lead white/egg/ linseed oil Fresh	Lead white/egg/ linseed oil Aged
Hydroxyl O-H stretching vibration due to lead white			3529.5 w	3527.6 w
Oxidation products				
O-H stretch due to water absorption				
N-H stretch	3288.4 m	3288.4 m	3288.4 w	3288.4 w
Amide II overtone	3080 w	3080 w	3080 vw	-
Olefinic stretching band [(=C-H)stretch]			3008.7 sh	3008.7 wsh
Lower concentration of unsaturated chains	3006 w	3006 w		
CH_2 stretch	2925.8	2925.8 s	2927.7 s	2927.7 s
CH_2 stretch	42854.5	2854.5 m	2856.4 m	2856.4 m
Trygliceride ester linkage	1745.5 m	1741.6 m	1741.6 s	1741.6 s
Amide I [C=O stretch]	1652.9 m	1646.8 m		
(-HC=CH-)stretch			1654.8 w	1635.5 w
Amide II	1548.7 m	1546.8 m	1542 sh	1542.9 w
C=C aromatic and conjugated	1460 msh	1460 msh		
anti-symmetric CO_3 stretching vibration	1390 m	1394.4 m	1396.4 vs	1396.4 vs
C-N stretch of primary amides				
Triglyceride ester linkage	1240.1 w	1244 w	1245.9 sh	1245.9 sh
Triglyceride ester linkage	1163 m	1163 w	1166.9 w	1166.9 w
Triglyceride ester linkage	1095.5 w	1095.5 vw	1099.3 w	1099.3 w
symmetric CO_3 stretching vibration			1047.3 w	1045.3 w
Aromatic C-H out-of-plane	970 w	-	977.8 w	979 w
Absorbance due to lead white	839 w	842.8 vw	835.1 w	835.1 w
Absorbance due to lead white	779 w	779 w	775 w	775.8 w
Lower concentration of unsaturated chains			729 w	730 w
CO_3 rocking deformation due to lead white	680.8 w	677 w	680.8 m	682.8 m
Hydrogen-bonded, out-of-plane O-H bend				

Red lead and binders: Red lead affects the spectrum of egg tempera quite significantly. Characteristic bands for the protein (Table 4.11), such as the triglyceride ester linkages have been masked by the red lead and this has resulted in a significant reduction in intensity. Absorption bands such as 970cm^{-1} and 720cm^{-1} have almost disappeared.

Likewise, it can be seen (Table 4.4.2.6) that red lead has affected the spectrum of egg yolk-linseed oil. Characteristic bands for the protein, such as the triglyceride ester linkages have been masked by the red lead and this has resulted to a significant reduction in intensity. The absorption band of 970cm^{-1} has almost disappeared. It can be observed in this spectrum as well, that the characterisation of protein was very difficult in the presence of oil.

Table 4.11 Red lead and binders

Interpretation	Red lead/ egg tempera Fresh	Red lead/ egg tempera Aged	Red lead/egg/ linseed oil Fresh	Red lead/egg/ linseed oil Aged
Oxidation products				
O-H stretch due to water absorption	3425 m	3425 m	3427.3 m	3429.2 s
N-H stretch	3276.8 m	3284.5 m		
Amide II overtone	3080 vw	3080 vw	3080 vw	3080 vw
Olefinic stretching band [(=C-H)stretch]			3008.7 w	3008.7 vw
Lower concentration of unsaturated chains	3006 vw	3006 vw		
CH_2 band	2923.9 s	2923.9 s	2925.8 s	2923.9 s
CH_2 band	2852.5 m	2852.5 m	2854.5 m	2854.5 m
Triglyceride ester linkage	1741.6 m	1735.8 m	1745.5 s	1741.6 m
Amide I [C=O stretch]	1650 wsh	1650 wsh		
Triglyceride ester linkage			1649 w	1643.2 m
(-HC=CH-)stretch	1637.5 m	1637.5 m	1548 w	1546.8 w
Amide II	1548 m	1542.9 m		
C=C aromatic and conjugated	1458 w	1455 w	1460 m	1456.2 m
anti-symmetric CO_3 stretching vibration	1409 wsh	1410 wsh		
C-N stretch of primary amides	1388.7 m	1388.7 m	1377.1 w	1400.2 m
Triglyceride ester linkage	1242.1 w	1242 vw	1236.3 w	1242.1 w
Triglyceride ester linkage	1161.1 w	1163 w	1164.9 m	1163 m
Triglyceride ester linkage	1099.3 w	1099 vw	1097.4 w	1101.3 w
Aromatic C-H out-of-plane	970 vw	-	970 vw	970 vw
Lower concentration of unsaturated chains	720 w	721.3 w	721.3 w	723.3 w
Absorption band due to red lead	685 vw	685 vw	680.8 w	673.1 w
Lower concentration of unsaturated chains				528.5 m
Hydrogen-bonded, out-of-plane O-H bend				434 m
Absorption band due to red lead	526.5 w	528.5 w	528.5 m	
Absorption band due to red lead	439.7 m	439.7 m	434 m	

All data are in wavenumbers (cm^{-1})

The following materials were not included as reference materials. However, since their presence was necessary for the completion of the database, the absorption bands were collected from published sources (Ioakimoglou, 1993).

Ground

Ground layer has the following characteristic bands in the mid infrared region: 2260.2 cm^{-1}, 1620 cm^{-1}, 1143 cm^{-1}, 1120 cm^{-1}, 668.81 cm^{-1} and 604.2 cm^{-1}

Animal glue

2921.4 cm^{-1}, 1646.6 cm^{-1}, 1631.9 cm^{-1}, 1518.6 cm^{-1} and 1451 cm^{-1}

Casein

1536.5 cm^{-1}, 1399 cm^{-1} and 1335.5 cm^{-1}

Observations: It can be observed that both egg yolk and oils have characteristic bands, which help them to be easily detected. However, when in form of emulsion, it was quite difficult to detect the egg. The carboxylic features from the oils have altered the intensities of absorbance concerning amide features. The presence of egg can be demonstrated by the absorptions at around 3289, 3080, 1660 and 1632 cm^{-1}, which seem to be quite durable.

It was also observed that all the three oils tested, have almost identical absorption bands, which make their characterization almost impossible with FTIR. It was easy to identify the presence of drying oils in the spectrum, but it was literally impossible to identify which oil is present.

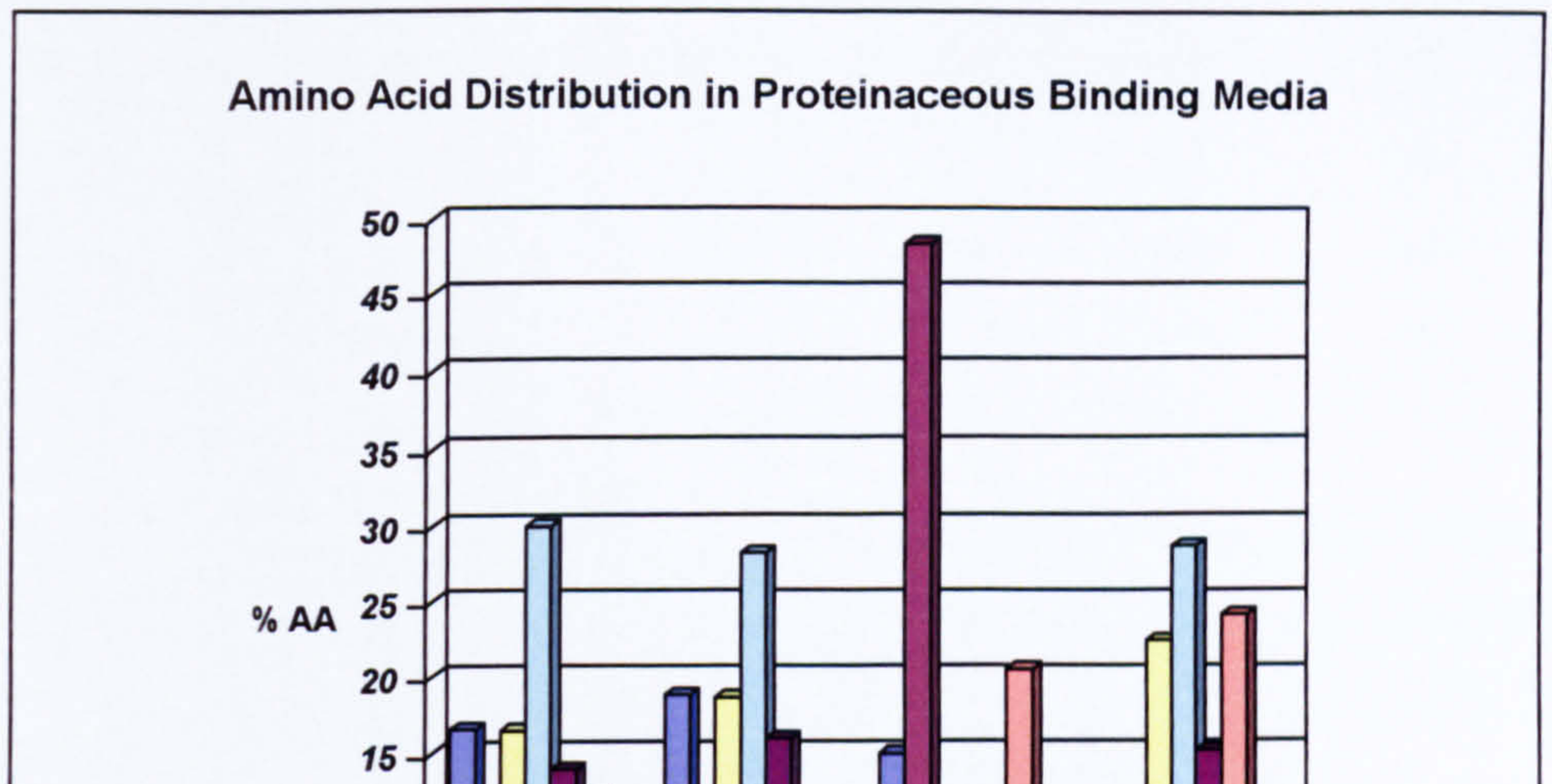
4.4.3 Gas Chromatography

The interpretation of the results from the analysis of binding material may be difficult due to the possible complex nature of the binders used. These may include proteinaceous materials (animal glue, egg and milk), drying oils (linseed, poppy, walnut oil) and/or natural resins (turpenoids) and starches or indeed combinations of examples from within these groups, for example an

egg-oil emulsion. A further difficulty arises from the contribution to the fatty acids resulting from both egg and milk components if present. Within this complexity two observations can be very diagnostic: (a) the presence of hydroxyproline confirms the use of animal glue which will also show a G/A ratio >2.5; (b) the absence of any amino acid confirms the use of a drying oil.

This section reports data derived from aged samples produced from single component binders and from simple emulsions in order to provide reference data for the subsequent analyses and also to provide a validity check by comparison with other published data. The reference results obtained for the seven stable amino acid concentrations are reported in Figure 4.2. As it can be seen, the relative abundances of the seven amino acids are so distinct for each medium, that they can be used to identify the type of the binder. Analytically, for egg yolk, 5 of the amino acid peak areas are similar (10 – 17 %) with Leucine at 30% and the total absence of hydroxyproline. Animal glue, on the contrary, contains Hydroxyproline, which is absent from the other proteins tested and high proportions of Alanine (15%), Glycine (48%) and Proline (21%). Finally, the major amino acids in casein are Leucine (29%), Proline (21%) and Valine (22%).

Figure 4.2 Distribution of the relative percentage content of the seven "stable" amino acids (A, G, V, L, I, P and OH-P) in the proteinaceous binding media reference samples



	Egg Yolk	Albumin	Gelatin	Casein
A	16,6	18,81	14,98	7,65
G	10,8	10,54	48,37	3,47
V	16,5	18,64	4,29	22,39
L	30	28,24	5,1	28,66
I	14	15,98	4,73	15,18
P	12,1	9,76	20,5	24,11
OH	0	0	2	0

However, in mixed systems relative abundance may not be sufficient on its own for identification. Thus, the use of ratios can help those situations (Table 4.12). The presence of Hydroxyproline can be used as a mark for gelatin (animal glue) since it is not detected in any other protein, while the major amino acids detected in this medium are Alanine, Glycine and Proline. Furthermore, gelatin shows a G/A ratio >2.5, as reported by Castro *et al.* (1997). On the other hand, for egg yolk, the relative abundances for 5 of the amino acids are similar, with Leucine in excess. Casein presents high proportions of Valine, Leucine and Proline and a P/A ratio >2.5 (Castro *et al.*, 1997).

Table 4.12 The relative amino acid percentage contents of the reference samples. A: alanine; G: glycine; V: valine; L: leucine; I: isoleucine; P: proline; OH-P: hydroxyproline

Sample	A	G	V	L	I	P	OH-P
Egg yolk aged	1	0.6	0.9	1.4	0.7	0.6	0
Albumin	1	0.6	1.0	1.5	0.9	0.6	0
Gelatin	1	3.0	0.3	0.4	0.3	1.4	0.2
Casein	1	0.5	3.0	4.0	2.0	3.0	0

The results in Table 4.13 compare the ratios of the fatty acids C9/C16, C18:1/C18 and C16/C18 for the range of reference materials prepared. It was stated earlier (Chapter 3) that egg yolk would contribute to the fatty acid constituents in an emulsion and it is interesting therefore to compare the combined results from the single media with those from the emulsion assuming that the overall contribution is half the sum of both contributions for a 1:1 mixture. This comparison shown by the data in Table 4.13, shows good agreement for both C9/C16 and C16/C18 ratios, whereas there are significant differences between the calculated and experimental data for the C18:1/C18 ratios.

As it can be observed in Table 4.13 for fresh egg yolk the ratio C16/C18 was 3.2 ± 0.3 while for aged egg yolk the ratio C16/C18 was around 2.5 ± 0.1. Accordingly, for raw linseed oil the ratio C16/C18 is 1.6 ± 0.1, while for egg/linseed oil emulsion, the ratio of C16/C18 was 1.8 ± 0.4. The C16/C18 ratios for the aged samples of both cold-pressed linseed oil and stand oil gave values lower than that obtained for raw linseed oil. Analytically, for aged cold-pressed linseed oil the C16/C18 ratio was 1.4 ± 0.1, while the C16/C18 ratio for aged stand oil was 1.2 ± 0.1. For walnut oil the C16/C18 ratio was around 3.3 ± 0.1, while for egg/walnut oil emulsion, the ratio of C16/C18 was around 2.9 ± 0.1. Finally, for poppy oil the C16/C18 ratio was 3.9 ± 0.1, while for egg/poppy oil emulsion, the ratio of C16/C18 was around 4.9 ± 1.4.

Table 4.13 The Fatty Acid relative ratios of the reference binding media

Sample	C9/C16	C18:1/C18	C16/C18
Poppy oil aged T°	0.4 ± 0.05	1.1 ± 0.1	3.9 ± 0.1
Walnut oil aged T°	0.7 ± 0.1	0.5 ± 0.05	3.3 ± 0.1
Linseed oil aged T°	1.0 ± 0.1	1.1 ± 0.1	1.6 ± 0.1
Stand oil aged T°	0.3 ± 0.1	2.1 ± 0.1	1.2 ± 0.1
Cold-pressed linseed oil aged T°	1.0 ± 0.1	1.8 ± 0.1	1.4 ± 0.1
Egg yolk fresh	0.03 ± 0.02	2.8 ± 0.8	3.2 ± 0.3
Egg yolk aged T°	0.17 ± 0.02	2.2 ± 0.2	2.0 ± 0.1
Egg yolk aged T° + UV	0.07 ± 0.02	0.16 ± 0.2	2.4 ± 0.1
Egg tempera aged T°	0.05 ± 0.02	0.6 ± 0.2	2.5 ± 0.1
Egg yolk – Poppy oil aged	0.2 ± 0.05	0.4 ± 0.02	4.9 ± 1.4
Egg yolk – Walnut oil aged	0.3 ± 0.1	0.4 ± 0.1	2.9 ± 0.1
Egg – Linseed oil aged	0.5 ± 0.2	0.2 ± 0.05	1.8 ± 0.4

The reference data from Tables 4.12 and 4.13 combined with information from the literature was used to analyse the 201 results obtained from 121 icons presented in Chapter 5. These results are the mean values from at least three determinations and are subject to an uncertainty of 5 percent. The analysis was further complicated by the presence of ground material in the samples which contributed to the amino acid levels. The determination of the species present was therefore derived from a careful consideration of the ratios of the amino acids, the presence of hydroxyproline and the fatty acid ratios C9/C16 and C16/C18.

4.4.4 Post-Byzantine icons for analysis

In this section the icons studied are presented. Due to the high number of icons they are presented in tabular format based on the school concerned (Tables 4.14 – 4.17). The photos of the icons are displayed in Appendix IV.

Table 4.14 Icons studied from the Cretan School

No	Subject	Code	Artist	Date	Provenance	Location
1.	Staurosis	BXM981	Unknown	14th c.	Monemvasia	Byzantine Christian Museum - Athens
2.	St Theodoros the Tiron	SL285/L335	Aggelos Akotantos	1st half of 15th c.	Crete, Constaninople	Byzantine Christian Museum - Athens
3.	St John the Baptist	1551/T2639	Aggelos Akotantos	1st half of 15th c.	Crete, Constaninople	Byzantine Christian Museum - Athens
4.	The entrance of Virgin Mary	Lo209/SL208	Aggelos Akotantos	1st half of 15th c.	Crete, Constaninople	Byzantine Christian Museum - Athens
5.	Virgin Mary the Kardiotissa	1552/T1582/L367	Aggelos Akotantos	1st half of 15th c.	Crete, Constaninople	Byzantine Christian Museum - Athens
6.	St George	-	Aggelos Akotantos	2nd quarter of 15th c.	Crete, Constaninople	Byzantine Christian Museum - Athens
7.	The Crucifixion	-	Andreas Pavias	2nd half of 15th c.	Crete	National Gallery/Alexandros Soutzos Museum -Athens
8.	Virgin Mary with St Catherine	-	Aggelos	Early 16th c.	Crete	Monastery of Patmos - Cyclades
9.	Virgin Mary holding Jesus Christ (Veneziana)	-	Unknown	~1540	Venice?	Monastery of Patmos - Cyclades
10.	Christ the Great Archpriest	L212	Michael Damaskinos	2nd half 16th c.	Crete or Venice ?	Byzantine Christian Museum – Athens [Lomverdou Collection]
11.	The life of Christ	-	Georgios Klontzas (?)	2nd half 16th c. – early 17th c.	Crete	Byzantine Christian Museum - Athens
12.	Christ the Great Archpriest	1968/a/a22	Unknown	17th	Crete ?	Academy of Athens
13.	St Nicolaos, Vasileios & Antonios	-	Unknown	17th	Crete	Academy of Athens
14.	Virgin Mary Enthroned	T362	Emmanuel Tzanes	1661	Venice?	Byzantine Christian Museum – Athens [Makkas Collection]
15.	Epi soi chairei	-	Theodoros Poulakis	1660-1690	Crete	Monastery of Patmos - Cyclades
16.	The draught of Joseph in the pit and his sale to the Israelis	BEI 959	Theodoros Poulakis	2nd half of 17thc.	Crete - Corfu or Venice ?	Museum of Byzantine Culture – Thessaloniki [Kaftatzoglou Collection]

No	Subject	Code	Artist	Date	Provenance	Location
17.	The moaming of Jacob for the death of Joseph	BEI 957	Theodoros Poulakis	2nd half of 17thc.	Crete - Corfu or Venice ?	Museum of Byzantine Culture – Thessaloniki [Kaftatzoglou Collection]
18.	The sale of Joseph to Pentefris in Egypt	BEI 958	Theodoros Poulakis	2nd half of 17thc.	Crete - Corfu or Venice ?	Museum of Byzantine Culture – Thessaloniki [Kaftatzoglou Collection]
19.	Prophet Elias	BXM1576	Theodoros Poulakis	2nd half of 17thc.	Corfu - Ionion	Church of Ano Korakiana - Corfu
20.	Archangel Michael	26A	Theodoros Poulakis	2nd half of 17thc.	Crete - Corfu or Venice ?	Benaki Museum - Athens
21.	St Spyridon with Scenes of His Life	29	Theodoros Poulakis	Late 17th cent	Corfu ?	Benaki Museum - Athens
22.	The Hypapante	1968/a/a27	Unknown	Late 17th -early18thc.	Crete or Ionion Islands	Academy of Athens
23.	The palm carrier (Vaioforos)	1968/a/a?	Unknown	Late 17th -early18thc.	Crete or Ionion Islands	Academy of Athens

Table 4.15 Icons studied from the Heptanesian School – Ionian Islands

No	Subject	Code	Artist	Date	Provenance	Location
24.	The birth of Christ	1968/a/a26	Unknown	Late 17th c.	Ionion Islands	Academy of Athens
25.	Virgin Mary holding Crucified Christ	1968/a/a6	Unknown	Late 17th c.	Ionion Islands	Academy of Athens
26.	The symbol of Faith (part B)	1968/a/a4	Unknown	Late 17th c.	Ionion Islands	Academy of Athens
27.	The Epitaph Lament	1968/a/a12	Unknown	Late 17th c.	Ionion Islands	Academy of Athens
28.	The Crucifixion	1968/a/a29	Unknown	Late 17th c.	Ionion Islands	Academy of Athens
29.	Archpriest	-	Panayiotis Doxaras (?)	Late 17th c.	Corfu ?	Byzantine Christian Museum - Athens
30.	Allegory of the Holly Communion	SL190 / 191	Konstantinos Kontarinis (?) (1699 – 1732)	1st half 18th c.	Ionion Islands	Byzantine Christian Museum - Athens [Lomverdou Collection]
31.	Virgin Mary holding Baby Jesus	3033	Konstantinos Kontarinis (1699 – 1732)	1st half 18th c.	Ionion Islands	Benaki Museum- - Athens
32.	The worshipping of the Shepherds	147	Stephanos Tzankarolas (late 17th –early 18thc.)	Early 18th c.	Corfu? - Ionion	National Gallery/Alexandros Soutzos Museum - Athens
33.	The Paradise and the Hell	-	Unknown	Early 18th c.	Ionion Islands	National Gallery/Alexandros Soutzos Museum – Athens
34.	Jesus Christ washes the feet of His Disciples	-	Panayiotis Doxaras	Early 18th c.	Ionion – Zakynthos ?	Private Owner - Athens

Table 4.16 Icons studied from the Heptanesian School – Cephalonia

No	Subject	Code	Artist	Date	Provenance	Location
35.	Virgin Mary with Christ	-	Unknown	17th c. (?)	Cephalonia - Ionion	Chapel of Holly Virgin Mary "I kyria tis Argilou" (The Lady of the Argil), Kontogenada
36.	St John the Baptist	-	Unknown	17th c. (?)	Cephalonia - Ionion	Chapel of Holly Virgin Mary "I kyria tis Argilou" (The Lady of the Argil), Kontogenada
37.	Jesus Christ - Holy Gate	-	Unknown	17th c. (?)	Cephalonia - Ionion	Chapel of Holly Virgin Mary "I kyria tis Argilou" (The Lady of the Argil), Kontogenada
38.	Archangel Michael	-	Unknown	17th c. (?)	Cephalonia - Ionion	Chapel of Holly Virgin Mary "I kyria tis Argilou" (The Lady of the Argil), Kontogenada
39.	Pantokrator	-	Unknown	17th c. (?)	Cephalonia - Ionion	Chapel of Holly Virgin Mary "I kyria tis Argilou" (The Lady of the Argil), Kontogenada
40.	Archangel Gabriel	-	Unknown	17th c. (?)	Cephalonia - Ionion	Chapel of Holly Virgin Mary "I kyria tis Argilou" (The Lady of the Argil), Kontogenada
41.	The Assumption	-	Unknown	17th c. (?)	Cephalonia - Ionion	Chapel of Holly Virgin Mary "I kyria tis Argilou" (The Lady of the Argil), Kontogenada
42.	The Assumption	-	Unknown	~18th c.	Cephalonia - Ionion	Chapel of St John Theologos, Kontogenada
43.	Archangel Gabriel	-	Unknown	~18th c.	Cephalonia - Ionion	Chapel of St John Theologos, Kontogenada
44.	Jesus Christ	-	Unknown	~18th c.	Cephalonia - Ionion	Chapel of St John Theologos, Kontogenada
45.	St John Theologos	-	Unknown	~18th c.	Cephalonia - Ionion	Chapel of St John Theologos, Kontogenada
46.	St.... (cannot be distinguished)	27	Unknown	18th c.?	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
47.	St John the Baptist, St Spyridon, Maria Magdalene & St Gerasimos (?)	13	Unknown	18th c.?	Cephalonia - Ionion	Chapel of the Assumption, Oronghi

No	Subject	Code	Artist	Date	Provenance	Location
48.	The Assumption	21	Unknown	20th of May 1742	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
49.	The Annunciation	11	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
50.	The Nativity	6	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
51.	The palm carrier (Vaioforos)	10	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
52.	The Circumcision of Christ	2	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
53.	The Baptism of Christ	4	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
54.	The Raising of Lazarus	3	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
55.	The Resurrection of Christ	5	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
56.	The Last Supper	7	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
57.	The Hypapante	9	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
58.	The Pentecost	1	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
59.	The Ascension of Christ	8	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
60.	The Metamorphosis	12	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
61.	Mother of God	17	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
62.	Christ Pantokrator	18	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
63.	St John the Baptist	16	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
64.	Diptych of the Prothesis.	15	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
65.	St Vassilios	22	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
66.	St John Chrysostomos	20	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
67.	St Gregorios	19	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
68.	The Crucifixion	14	Unknown	Mid 18th c	Cephalonia - Ionion	Chapel of the Assumption, Oronghi
69.	St Gerasimos	-	Mourdelatos	1839	Cephalonia - Ionion	Chapel of St John Theologos, Kontogenada

Table 4.17 Icons studied from the Heptanesian School - Zakynthos

No	Subject	Code	Artist	Date	Provenance	Location
70.	St John the Baptist	MZ821	Michael Damaskinos	2nd half of 16th c.	Church of St Spyridon of Flampouriari	Post-Byzantine Museum - Zakynthos
71.	Gate with St Antonios	MZ5	Unknown	2nd half of 16th c.	Church of St Constantine of the Gardens	Post-Byzantine Museum - Zakynthos
72.	The Resurrection	-	Ilias Moskos	17th c.	Metropolitan Church	Ecclesiastical Museum - Zakynthos
73.	Jesus and John	MZ105	Unknown	17th c.	Church of St Spyridon of Flampouriari	Post-Byzantine Museum - Zakynthos
74.	The Preaching of John	MZ106	Unknown	17th c.	Church of St Spyridon of Flampouriari	Post-Byzantine Museum - Zakynthos
75.	St Dynati	-	Unknown	17th c.	Holly Monastery Strofadon	Ecclesiastical Museum - Zakynthos
76.	Archangel	-	Leo Moskos	17th c.	Skopiotissa	Post-Byzantine Museum - Zakynthos
77.	The Decollation of St John the Baptist	MZ115	Demetrios	2nd half of 17th c.	Church of St Marina	Post-Byzantine Museum - Zakynthos
78.	Metamorphosis	MZ11	Victor	1670	Old Museum	Post-Byzantine Museum - Zakynthos
79.	Christ the Great Archpriest	-	Panayiotis Doxaras	1691	Lady of the Angels	Ecclesiastical Museum - Zakynthos
80.	The Crucifixion	-	Panayiotis Doxaras	End of 17th c.	Holly Monastery Strofadon	Zakynthos
81.	The Adoration of the Shepherds	-	Panayiotis Doxaras	End of 17th c.	Holly Monastery Strofadon	Ecclesiastical Museum - Zakynthos
82.	The crossing of the Nile	MZ131	Unknown	End of 17th c.	Old Museum	Post-Byzantine Museum - Zakynthos
83.	Wooden icon-stand with St Demetrios & Scene of His Life	MZ57	Unknown	18th c.	Church of St Demetrios of Kolla	Post-Byzantine Museum - Zakynthos
84.	St Barbara & Scenes of Her Life	MZ114	Unknown	18th c.	Old Museum	Post-Byzantine Museum - Zakynthos
85.	Archangel Gabriel	MZ124	Unknown	18th c.	Old Museum	Post-Byzantine Museum - Zakynthos
86.	Archangel Michael	MZ125	Unknown	18th c.	Old Museum	Post-Byzantine Museum - Zakynthos
87.	Prophet Ionas	MZ132	Unknown	18th c.	Church of St Spyridon of Flampouriari	Post-Byzantine Museum - Zakynthos
88.	The Trinity and Archangels	-	Unknown	18th c.	Holly Monastery Strofadon	Ecclesiastical Museum - Zakynthos
89.	St John the Baptist	MZ94	Nikolaos Kallergis	1st quarter of 18th c.	Church of Virgin Mary of Tsouroufli	Post-Byzantine Museum - Zakynthos
90.	Christ the Great Archpriest	MZ93	Nikolaos Kallergis	1723	Church of Virgin Mary of Tsouroufli	Post-Byzantine Museum - Zakynthos
91.	The meeting of Mary and Elisabeth	-	Stentas ?	1723	The Lady of the Angels	Zakynthos

No	Subject	Code	Artist	Date	Provenance	Location
92.	The decollation of St John the Baptist	-	Stentas ?	1723	The Lady of the Angels	Zakynthos
93.	St Demetrios	MZ40	Unknown	1730		Post-Byzantine Museum - Zakynthos
94.	Christ upheld by an angel	MZ120	Unknown	1732	Church of Virgin Mary of Tsouroufli	Post-Byzantine Museum - Zakynthos
95.	Angel	MZ121	Unknown	1732	Church of Virgin Mary of Tsouroufli	Post-Byzantine Museum - Zakynthos
96.	The entrance of Virgin Mary	MZ389	Nikolaos Kallergis	1739	Church of the Holly Spirit Gaitaniou	Post-Byzantine Museum - Zakynthos
97.	Angel with the Symbols of Passion	MZ891	Unknown	Early 18th c.	Vestry's gate	Post-Byzantine Museum - Zakynthos
98.	St John the Baptist	-	Unknown	1st half of 18th c.	Church of St John Gkremnon	Ecclesiastical Museum - Zakynthos
99.	St John the Baptist	MZ39	Unknown	Mid 18th c.		Post-Byzantine Museum - Zakynthos
100.	Prophet	MZ538	Nikolaos Doxaras or Stauros Pazigetis	Mid 18th c.	Church of Virgin Mary Faneromeni	Post-Byzantine Museum - Zakynthos
101.	The Litany of St Dionysios	-	Nikolaos Koutouzis	1766	Holly Monastery Strofadon	Ecclesiastical Museum - Zakynthos
102.	Christ the Great Archpriest	MZ387	Demetrios Stavrakis	1770		Post-Byzantine Museum - Zakynthos
103.	Jesus Christ and the Prophets 1	-	Nikolaos Koutouzis	End of 18th c.	Holly Monastery Strofadon	Ecclesiastical Museum - Zakynthos
104.	Jesus Christ and the Prophets 2	-	Nikolaos Koutouzis	End of 18th c.	Holly Monastery Strofadon	Ecclesiastical Museum - Zakynthos
105.	The lamb	-	Nikolaos Koutouzis	End of 18th c.	Holly Monastery Strofadon	Ecclesiastical Museum - Zakynthos
106.	St Dionysios	-	Unknown	End of 18th c.	Holly Monastery Strofadon	Ecclesiastical Museum - Zakynthos
107.	The Dinner at the Emmaous		Nikolaos Koutouzis	End of 18th c.	Holly Monastery Strofadon	Ecclesiastical Museum - Zakynthos
108.	The Annunciation	MZ191	Nikolaos Koutouzis	End of 18th c.	St Spyridon of Flampouriari	Post-Byzantine Museum - Zakynthos
109.	The Lamb	MZ801	Unknown	End of 18th c.	Old Museum	Post-Byzantine Museum - Zakynthos
110.	Ecce Hommo!	MZ486	Unknown	End of 18th c. beginning of 19th c.		Post-Byzantine Museum - Zakynthos
111.	The Annunciation	MZ608	Unknown	End of 18th c. beginning of 19thc.		Post-Byzantine Museum - Zakynthos
112.	The Lamb	MZ815	Unknown	End of 18th c. beginning of 19thc.	St Antonios of Andritsi	Post-Byzantine Museum - Zakynthos
113.	The Worshipping of the Shepherds	-	Unknown	End of 18th c. beginning of 19thc.	Holly Monastery Strofadon	Ecclesiastical Museum - Zakynthos

No	Subject	Code	Artist	Date	Provenance	Location
114.	The Baptism	MZ148	Nikolaos Kantounis	Beginning of 19th c. (σ. 340)	Church of St Anargyri	Post-Byzantine Museum - Zakynthos
115.	The Deposition	MZ150	Nikolaos Kantounis	Beginning of 19th c. (σ. 344)	Church of St Anargyri	Post-Byzantine Museum - Zakynthos
116.	St Gregorios the Theologian	MZ160	Nikolaos Kantounis	Beginning of 19th c. (σ.378)	Church of St George ton Kalogreon	Post-Byzantine Museum - Zakynthos
117.	Christ on the Cross	MZ175	Unknown	Beginning of 19th c. (σ.422)	Church of Virgin Mary the Episkopiany	Post-Byzantine Museum - Zakynthos
118.	Christ on the Cross on wooden-carved base	MZ139/140	Nikolaos Koutouzis	Beginning of 19th c. (σ.358)	Church of Virgin Mary Faneromeni	Post-Byzantine Museum - Zakynthos
119.	The Lamb	MZ757	Unknown	Early 19th c.	-	Post-Byzantine Museum - Zakynthos
120.	Evangelist Lucas is painting Virgin Mary	MZ196	Nikolaos Kantounis	1833 (σ.460)	Church of Virgin Mary Pikridiotissa	Post-Byzantine Museum - Zakynthos
121.	The Annunciation	MZ224	Stauros Pelekasis	19th c.	Zakynthos - Ionion	Post-Byzantine Museum - Zakynthos

CHAPTER 5

RESULTS - DISCUSSION

5.1 Results

The samples were analysed as detailed in chapter 4 and due to the large quantity of data generated the methods used for each sample and the results obtained have been summarised in a series of spreadsheets (5.1 – 5.4). As can be seen from the spreadsheets for some icons, more than one sample was collected. GC analysis was not performed on all of them due to the lack of adequate sample quantity. In those cases, spectroscopic analysis was used which as was stated in Chapter 4, cannot offer positive identification of the binding medium present, especially in cases of emulsions. This fact is pointed out by the question mark at the emulsion section of the spreadsheet of results.

The amino acid (AA) and fatty acid (FA) ratios from the reference samples along with data from literature sources were used to analyse the results from the GC component of this research as detailed in the section following the spreadsheet for each individual school.

In cases where animal glue and a drying oil were detected in the same paint sample, there are questions as to whether this was an emulsion:

- the samples may have been a mixture of both ground and pigment layer
- this may be an oil based layer over a proteinaceous layer
- there is no literature basis for such emulsions

Thus, in the emulsion section, the **N*** indicates this particularity. This is not valid for ground samples since, both Cennini (1954) and ek Fourna (1906) refer to the use of mixed ground layer.

Table 5.1 Results from icons studied from the Cretan School

Icon	Samples	Pigments				Binding medium									Analytical Techniques				
						Protein				Drying Oil				Emulsion					
		Lead white	Cinnabar	Red lead	Other	Egg yolk	Animal glue	Casein	unidentified	Linseed oil	Walnut oil	Poppy oil	unidentified	Y/N	SEM/EDX	µFT-IR	GC	*Raman*	*Cross-sections*
	1-green				terre verte	x					x			Y	x		x		
1	1-blue				egyptian blue, ultramarine, Pb	x					x			Y	x		x		
2	2-grey					x								N			x		
3	3-grey					x								N			x		
4	4-red					x								N			x		
5	5-light blue					x								N			x		
6	6-red					x								N			x		
7	7-white	x					x	x						N?	x	x		x	
	7-red		x				x	x		x				Y	x	x	x	x	
8	8-red		x	x						x				N	x	x	x		
9	9-red 1		x	x		x	x							N	x	x	x		
	9-red 2		x	x		x	x							N	x	x	x		
10	10-white	x				x								N	x	x	x		
	10-red		x			x	x			x				Y	x	x	x		
11	11-red		x	x			x			x				N*	x	x	x		
	11-white	x			Fe, Si					x				N?	x	x			
12	12-white	x											x	N	x	x			
	12-red		x								x			N	x	x	x		
	12-gesso						x				x			Y			x		
13	13-white	x				x								N	x	x		x	
	13-red		x			x	x					x		Y	x	x	x	x	
14	14-red 1			x		x	x			x				Y	x	x	x		

Icon	Samples	Pigments				Binding medium									Analytical Techniques				
						Protein				Drying Oil				Emulsion					
		Lead white	Cinnabar	Red lead	Other	Egg yolk	Animal glue	Casein	Unidentified	Linseed oil	Walnut oil	Poppy oil	Unidentified	Y/N	SEM/EDX	µFT-IR	GC	*Raman*	*Cross-sections*
15	15-red		x	x		x	x							N	x		x		
16	16-white	x				x								N	x	x	x		
17	17-white	x					x			x				N*	x	x	x	x	
18	18-white	x				x	x				x			Y	x		x		
19	19-white	x				x	x							N	x	x	x		
20	20-white	x				x								N?	x	x			
	20-red		x	x		x					x			Y	x	x	x		
21	21-white	x				x				x				Y	x	x	x		
	21-red			x		x								N?	x	x			
22	22-red	x	x	x		x	x							N?	x	x		x	
	22-grey					x	x			x				Y		x	x		
	22-gesso				$CaSO_4$	x	x				x			Y			x	x	
23	23-red		x			x	x							N	x	x	x	x	
	23-gesso						x			x				Y			x		

5.1.1.1 Summary of analyses: Icons 1 – 23

1.	blue green	Both samples were quite hydrolysed. The AA values were indicative for egg yolk and the FA ratios for walnut oil.
2.	grey	Egg yolk was detected. The FA ratios and especially, the C16/C18 gave the value of 2.36, which along with the AA distribution and the exceeding of L confirm the use of egg as a binder.
3.	grey	The GC analysis of the sample showed that the binder has suffered a high degree of hydrolysis. Therefore the characterisation of the type of medium cannot be made with safety. However, the presence of some AA and their distribution confirm the use of a proteinaceous medium, possibly egg yolk.
4.	red	Very hydrolysed sample. With difficulty some AA can be detected. The presence of C16 and C18 and their ratio (2.33) suggests the use of egg yolk. However, this cannot constitute a safe characterisation of the organic binding medium.
5.	light blue	Egg yolk was detected. The FA ratios and especially, the C16/C18 gave the value of 2.53, which along with the AA distribution confirm the use of egg as a binder.
6.	red	The GC analysis of the sample showed that the binder has suffered a high degree of hydrolysis. Therefore the characterisation of the type of medium is not possible. However, the distribution of the AA suggests the use of egg yolk.
7.	red	The interpretation of this sample was quite complicated. μFT-IR analysis showed the presence of gesso (2260.2 – 1620 – 1143 – 1120 – 668.81 – 604.2 cm^{-1}), animal glue (2921.4 – 1646.6 – 1631.9 – 1518.6 – 1451 cm^{-1}) and casein (1536.5 – 1399 – 1335.5 cm^{-1}). GC analysis

		verified these results: Proteinaceous binder was detected but its characterisation proved quite difficult. The AA ratios suggested the use of animal glue and casein, while the FA ratios suggested the presence of linseed oil.
	white	The μFT-IR showed the presence of egg yolk (2923.1 – 2852.8 – 1385.2 – 1361.7 – 719.5 cm^{-1}), animal glue (2878 – 1632.1 1398.5 cm^{-1}), gesso (1143 – 1128.2 – 667.4 cm^{-1}) and lead white (1398.5 – 838.2 cm^{-1}). Linseed oil was not detected but even if it was present in the form of emulsion, it could not be detected with safety due to the overlapping of the absorption peaks, as it has been mentioned in Chapter 4. The question mark in the *Emulsion* section of the Result-spreadsheet μRaman detected only the presence of the pigments in both samples.
8.	red	Linseed oil.
9.	red1	The results of both samples are quite interesting since it
	red2	seems that the artist used a combination of red lead Pb_3O_4 mixed with cinnabar, HgS. The FT-IR analysis showed the presence of egg yolk (2922.8 – 2852.6 – 1733.9 – 1653 – 1636.2 – 1541.2 – 1457.9 – 1385.1 – 1244 – 968.9 – 669.2 cm^{-1}), and gesso (1618.2 – 1143.7 – 1115.9 cm^{-1}). The GC chromatograms of the samples present significant similarities with the one of egg yolk standard. The amino acid ratios and especially the G/A: 1 and the detection of OH-P suggest the presence of animal glue, possibly as a contamination from the ground layer. However, the ratios of the fatty acids approach the ones of the egg yolk standard, confirming, thus, the presence of egg yolk as binding medium.
10.	white	The AA and FA ratios suggest the use of egg yolk.
	red	The AA ratios suggest the presence of egg yolk, but the

		presence of OH-P confirm animal glue. The FA ratios show the use of emulsion with linseed oil.
		µFT-IR detected the presence of egg yolk.
11.	red	µFT-IR suggested the use of linseed oil (2920.1 – 2854.5 – 1462 – 1418.5 – 1251.5 – 729.4 cm^{-1}), animal glue (1652.2 – 1551.8 – 1436.5 – 1317.5 – 1232.8 – 1208.3 – 1050 cm^{-1}) and read lead (1407.4 cm^{-1}). The GC analysis verified those results.
	white	The FT-IR microscopy showed the presence of linseed oil (2923.1 – 2852.8 – 1632.1 – 1385.2 – 719.5 – 667.4 cm^{-1}), gesso (667.4 – 1128.2 cm^{-1}) and lead white (1398.5 – 838.2 cm^{-1}).
12.	white	µFT-IR showed the presence of a drying oil (2861.5 – 1729.1 – 1641.3 – 679.2 cm^{-1}) and lead white (3529.3 – 1405.7 – 1052.7 – 782.6 – 839.3 cm^{-1}). The identity of the oil present cannot be characterised by IR spectroscopy due to the identical absorption bands.
	red	GC analysis detected the use of walnut oil.
	gesso	Animal glue and walnut oil.
13.	white	µFT-IR showed the presence of egg yolk (2936.3 – 2854.5 – 1733 – 1651.1 – 1107.3 – 991.7cm^{-1}) and lead white (3533.1 – 1415.3 – 1063.9 – 847 – 688.5cm^{-1}). If a drying oil was present, it could not be detected.
	red	GC indicated the use of egg yolk with poppy oil and animal glue.
14.	red	The AA distribution and ratios suggest the use of animal glue (presence of OH-P) and the high percentage of L suggest the possible presence of egg yolk. Additionally, the FA ratio, especially, the C16/C18=1.9 suggest the presence of linseed oil, thus the use of an emulsion.
15.	red	The AA ratios and the presence of OH-P suggest the use of animal glue. However, the FA ratio of C9/C16: 0.1 and

		C16/C18: 2.1 confirm the use of egg yolk.
16.	white	The sample is seriously hydrolysed. The AA can be hardly detected but the FA ratios indicate the presence of egg yolk.
17.	white	μFT-IR indicated the presence of drying oil (2840.4 – 1672.2 – 1379.1 – 1165.6 - 975 cm^{-1}) and animal glue (1204 – 921.3 cm^{-1}). The GC analysis showed the presence of animal glue and linseed oil. μRaman indicated the use of lead white only.
18.	white	This sample was quite difficult to interpret. The presence of OH-P confirms the use of animal glue. However, the high percentage of I (3.1) could also be indicative for egg yolk. High percent of I is not characteristic for any type of protein. However, since I is an isomer of L, this type of phenomenon could occur during derivatization process. Finally, the FA ratio of C16/C18:3.4 suggests walnut oil as well.
19.	white	The FA and AA profile of the sample clearly shows the use of animal glue and egg yolk.
20.	red	The presence of egg yolk was detected at the 3296.6/3286.2 cm^{-1}, 3084.5 cm^{-1}, 1656.9/1651.7cm^{-1} and 1543.1/1537.9cm^{-1} bands with FT-IR microscopy. Similarly, the presence of red lead was detected at the 1424.1 and 710.3/715.5cm^{-1} bands. As cinnabar does not produce a spectrum at the mid-infrared region therefore it could not be detected. Finally, no bands leading to the presence of oil were detected. Gas chromatographic analysis through the FA and AA ratios of the sample clearly indicated the use of egg yolk and walnut oil.
	white	The sample was identified as lead white-egg tempera by μFT-IR. The main absorption bands that indicate the presence of egg were detected: 3296.6/3286.2cm^{-1},

		1651.7/1656.9cm^{-1} and 1527.6/1537.9cm^{-1}. The Amide II overtone band has disappeared, as well as the 1240cm^{-1} band for the triglyceride ester linkage. A shift of some absorption bands was noticed but this can be due to the age of the sample. If oil is present, it could not be detected, since there is no absorbance band that would lead to its characterisation.
21.	white	The sample was clearly identified by FT-IR microscopy as lead white-egg tempera. The main absorption bands that indicate the presence of egg were detected: 3296.9cm^{-1}, 3080cm^{-1}, 1651.7/1656.9cm^{-1} and 1532.8cm^{-1}. However, if oil is present, it could not be detected, since there is no absorbance band that would lead to its characterisation. A shift of some absorption bands was noticed but this could be due to the age of the sample. GC analysis and the FA and AA profile of the sample clearly indicated the use of egg yolk and linseed oil.
	red	µFT-IR detected the use of red lead at the 1408.6/1424.1cm^{-1} absorption bands and possibly at the 731/710.3cm^{-1} absorption bands. Egg yolk (3290 - 3080 - 2929.3 - 2862.1 - 1713.8 - 1656.9 – 1522 - 1455.2 - 1408.6 - 1243.1 - 1170.7 – 1099 cm^{-1}) was clearly identified, but the presence of the oil, if any could be detected.
22.	red	The µFT-IR analysis of this sample indicated the use of egg yolk and animal glue (3286.4 – 2857.2 – 2926.9 – 1653.6 – 1634.3 – 1535.1 – 1411 – 732.9 – 1060.8 cm^{-1}). If a drying oil is present it cannot be detected due to the overlapping of the absorption bands.
	grey gesso	The GC results of the samples (Grey and Gesso) from this icon, present the possible use of emulsions in both the paint and ground layer. The grey paint layer seems to

		have been constructed by egg yolk and linseed oil, while the ground preparation layer with animal glue and poppy oil. Additionally, in the paint layer animal glue was detected, possibly as a contamination from the ground. μRaman of 22-gesso indicated the use of gypsum ($CaSO_4$).
23.	red	Egg yolk with animal glue.
	gesso	Animal Glue with linseed oil.

Table 5.2 Results from icons studied from the Ionian School

Icon	Samples	Pigments				Binding medium									Analytical Techniques				
						Protein				Drying Oil				Emulsion					
		Lead white	Cinnabar	Red lead	Other	Egg yolk	Animal glue	Casein	unidentified	Linseed oil	Walnut oil	Poppy oil	unidentified	Y/N	SEM/EDX	µFT-IR	GC	*Raman*	*Cross-sections*
24	24-white	x				x	x							N?	x	x			
	24-red			x		x	x			x				Y	x	x	x		
	24-gesso						x							N			x		
25	25-red		x			x	x							N		x	x		
	25-brown					x	x							N			x		
26	26-red		x	x		x	x						x	Y?	x	x	x		
	26-white	x				x	x			x				Y	x	x	x		
27	27-red		x			x	x				x			Y	x	x	x		
28	28-red		x			x	x							N?	x	x	x		
29	29-white	x				x		x		x				Y	x		x	x	
30	30-red			x		x	x			x				Y	x	x	x		
31	31-red		x			x				x				Y	x	x	x		
32	32-red		x	x		x								N	x	x	x		
	32-white	x				x								N?	x	x			
33	33-white	x							x					N?	x	x		x	
	33-red		x				x	x		x				N*	x	x	x	x	
34	34-white	x								x				N	x	x	x		x
	34-red		x	x						x				N	x	x	x		x

5.1.2.1 Summary of analyses: Icons 24 – 34

24.	white	μFT-IR microscopy indicated the use of egg yolk (3424.3 – 2925.6 – 2854.2 – 1727.8 – 1456.1 – 1365.8 cm^{-1}), animal glue (1526.1 – 1456.1 – 1426.9 cm^{-1}) and lead white (3535.5 – 1400.3 – 1049.2 – 837.7 - 683 cm^{-1}).
	red	GC detected egg yolk with linseed oil and animal glue possibly due to contamination from the ground layer.
	gesso	Animal glue only.
25.	red	Egg yolk and animal glue. The L and I did not derivatize as expected.
	brown	Egg yolk and animal glue.
26.	red	Egg yolk with animal glue (OH-P). The FA ratios lead to egg yolk (C16/C18: 2.6), but the slightly elevated value of C9/C16 (0.3) could suggest the presence of a drying oil (possibly walnut oil) not in 1:1 ratio.
	white	The GC analysis of this sample showed the presence of animal glue and an emulsion consisting of egg yolk and linseed oil. The AA present again the same phenomenon as in sample "16-white" with L and I. However, the overall profile point to animal glue and egg yolk. The FA ratios of C9/C16 (0.9) and C16/C18 (2) confirm the presence of linseed oil.
27.	red	Egg yolk and walnut oil and animal glue. The presence of animal glue may come from the ground layer.
28.	red	μFT-IR indicated the presence of egg yolk (2854.5 – 1670 – 1458.6 – 1251.7 – 1044.7 cm^{-1}), animal glue (2931.5 – 1670 – 1540.4 – 1415.3 cm^{-1}) and gesso (3408 – 2228.7 – 2175.8 – 1140.9 – 669.3 cm^{-1}). Cinnabar does not produce a spectrum at the mid-infrared region therefore it could not be detected. The GC analysis of the current sample, did not detect any amino acids or fatty acids. This

		could be either due to the high degree of hydrolysis, or to the non successful treatment of the sample.
29.	white	The interpretation of this sample was quite difficult. The amino acid ratio and the distribution due to the high value of V: 2 suggest the presence of casein, while the overall of the AA profile could suggest the presence of egg yolk as well. Casein could come from the ground layer. Finally, the FA ratios suggest the possible use of linseed oil.
30.	red	Egg yolk, animal glue and linseed oil.
31.	red	The medium used in this sample seems to be an emulsion of egg yolk and linseed oil.
32.	white	The sample was clearly identified by μFT-IR as lead white-egg tempera. The main absorption bands that indicate the presence of egg were detected: 3296.6cm^{-1}, 3080cm^{-1}, 1650/1660cm^{-1} and 1537.9/1532.8cm^{-1}.
	red	The interpretation of this sample was quite difficult. The two infrared spectra obtained from the same sample had some differences possibly due to the sample that was picked up. The first one seemed to contain more pigment than medium and this might be the reason for the absence of the 1740 and 1165cm^{-1} absorption bands, which characterise the triglyceride ester linkages. However, the presence of egg yolk was detected at the 3080 and 3006cm^{-1} bands. Similarly, the presence of red lead was detected at the 1424.1 and 710.3/715.5cm^{-1} bands. As it has been mentioned above, cinnabar does not produce a spectrum at the mid-infrared region therefore it could be detected. Finally, no bands leading to the presence of oil were observed. The results were verified by gas chromatography.
33.	red	The GC interpretation of this sample was quite difficult. Possibly the sample has undergone a strong hydrolysis

		process or it has been affected by previous restoration treatments. The AA and FA ratios indicate the use of casein, animal glue and linseed oil.
	white	The analysis of this sample by μFT-IR detected the lead white and the presence of a protein but it could not characterise the type of protein used. Additionally, as previously mentioned, if a drying oil was used, it could not be determined.
34.	white red	The physicochemical analysis of these samples showed that the medium used by the artist was a drying oil. This was also confirmed by staining of cross-sections. GC analysis showed that the oil used was linseed oil.

Table 5.3 Results from icons studied from Cephalonia

Icon	Samples	Pigments				Binding medium									Analytical Techniques				
						Protein				Drying Oil				Emulsion					
		Lead white	Cinnabar	Red lead	Other	Egg yolk	Animal glue	Casein	unidentified	Linseed oil	Walnut oil	Poppy oil	unidentified	Y/N	SEM/EDX	µFT-IR	GC	*Raman*	*Cross-sections*
35	35-paint								x				x	Y					x
	35-ground								x					N					x
36	36-paint								x				x	Y					x
	36-ground								x					N					x
37	37-paint								x				x	Y					x
	37-ground								x				x	Y					x
38	38-paint												x	N					x
	38-ground												x	N					x
39	39-paint												x	N					x
	39-ground								x					N					x
40	40-paint								x				x	Y					x
	40-ground								x				x	Y					x
41	41-paint								x					N					x
	41-ground								x					N					x
42	42-paint								x					N					x
	42-ground								x					N					x
43	43-paint								x				x	Y					x
	43-ground												x	N					x
44	44-paint																		x
	44-ground																		x
45	45-paint								x				x	Y					x
	45-ground												x	N					x
46	46-white	x				x	x			x				Y	x		x	x	
	46-ground				$CaSO_4$			x			x			Y	x		x	x	

Icon	Samples	Pigments				Binding medium									Analytical Techniques				
						Protein				Drying Oil				Emulsion					
		Lead white	Cinnabar	Red lead	Other	Egg yolk	Animal glue	Casein	unidentified	Linseed oil	Walnut oil	Poppy oil	unidentified	Y/N	SEM/EDX	µFT-IR	GC	*Raman*	*Cross-sections*
47	47-red			x	Ca	x						x		Y	x		x	x	
	47-ground				$CaSO_4$		x			x				Y	x		x	x	
48	48-white	x			Ca, massicot	x	x					x		Y	x		x	x	
49	49-red		x	x	Ca	x	x			x				Y	x		x	x	
50	50-red		x	x	Ca	x	x			x				Y	x		x	x	
	50-white	x			Ca	x	x			x				Y	x		x	x	
51	51-red		x	x		x	x			x				Y	x		x	x	
52	52-red		x	x	Ca	x	x							N	x	x	x	x	
	52-blue					x				x				Y			x		
53	53-red		x	x	Ca, massicot	x				x				Y	x		x	x	
54	54-red		x	x	Ca, massicot	x	x							N	x		x	x	
	54-white	x			Ca	x	x				x			Y	x		x	x	
55	55-red		x		Ca	x	x							N	x		x	x	
	55-blue					x				x				Y			x		
56	56-red		x	x	massicot	x	x							N	x		x	x	
	56-blue					x	x							N			x		
57	57-red		x		Ca	x	x			x				Y	x		x	x	
58	58-red		x		Ca	x								N	x		x	x	
59	59-red		x			x								N	x		x	x	
	59-orange		x	x	Ca	x								N	x		x	x	
	59-ground				$CaSO_4$		x			x				Y	x		x	x	
60	60-red		x	x	Ca	x				x				Y	x		x	x	
61	61-red		x	x	Ca	x					x			Y	x		x	x	

Icon	Samples	Pigments				Binding medium									Analytical Techniques				
						Protein				Drying Oil				Emulsion					
		Lead white	Cinnabar	Red lead	Other	Egg yolk	Animal glue	Casein	unidentified	Linseed oil	Walnut oil	Poppy oil	unidentified	Y/N	SEM/EDX	µFT-IR	GC	*Raman*	*Cross-sections*
62	62-red		x	x	Ca	x					x			Y	x		x	x	
63	63-white	x			Ca	x								N	x		x	x	
64	64-white	x				x	x					x		Y	x		x	x	
65	65-red		x	x	Ca	x								N	x	x	x	x	
66	66-white	x			Ca	x	x						x	Y	x		x	x	
67	67-red		x	x	Fe (earth)	x								N	x		x	x	
68	68-white	x			Ca	x						x		Y	x	x	x	x	
	68-blue					x						x		Y		x	x	x	
	68-yellow										x			N		x	x	x	
69	69-paint								x				x	Y					x
	69-ground								x					N					x

5.1.3.1 Summary of analyses: Icons 35 – 69

35.	cross-section	The ground layer was stained. The paint stained slightly as well. Proteins in both layers. The paint layer may contain an emulsion.
36.	cross-section	Paint-Emulsion. Ground-protein.
37.	cross-section	All layers were slightly stained indicating the possible use of an emulsion.
38.	cross-section	Oily ground and paint.
39.	cross-section	The ground layer (protein used) stained while the paint didn't (oil).
40.	cross-section	The reaction to the staining reagent suggests the use of protein and oil.
41.	cross-section	Proteinaceous medium has been used for both ground and paint layer.
42.	cross-section	Proteinaceous medium has been used for both ground and paint layer.
43.	cross-section	The staining of the ground suggests the use of protein while the paint of an emulsion.
44.	cross-section	Not successful preparation of the cross-section for staining.
45.	cross-section	The ground layer didn't not stained thus the use of an oily ground is being implied. A mixed technique seems to have been used for the paint layer consisting of a Proteinaceous layer and an oily one on top.
46.	ground	Casein (P/A ratio >2.5) and walnut oil (C16/C18 >3). Additionally, μRaman indicated the use of gypsum ($CaSO_4$) for the ground layer.
	white	Animal glue, egg yolk and linseed oil.
47.	ground	Animal glue and linseed oil (anticipated AA ratios for animal glue). As in sample 46, μRaman indicated the use of

		gypsum ($CaSO_4$) for the ground layer.
	red	AA ratios indicative of egg yolk and the C16/C18 ratio of 4.3 indicates poppy oil.
48.	white	Animal glue, egg yolk and poppy oil (C16/C18=4.3). μRaman detected the presence of massicot.
49.	red	OH-P indicates animal glue but the AA ratio (eg G/A=1.5) is too low for animal glue alone, therefore some egg yolk present in the sample. The C16/C18 ratio is 1.4 and not 2.5 expected for egg. Therefore linseed oil may be present. μRaman could not of any help as far the determination of the binders are concerned.
50.	red	As above.
51.	red	As above.
52.	blue	No OH-P. The AA ratios indicative of egg yolk, whilst C9/C16 and C16/C18 ratios suggest linseed oil also present.
	red	OH-P indicated animal glue and the C16/C18 confirms egg yolk present also.
53.	red	No OH-P. A mixture of egg yolk and linseed oil. μRaman detected massicot.
54.	white	Animal glue and egg yolk present but the value of 0.6 (C9/C16) and 2.9 (C16/C18) indicate the use of walnut oil.
	red	Animal glue and egg yolk. μRaman again, detected also the presence of massicot in the sample.
55.	blue	No OH-P but a high G/A ratio (2.1) suggests gelatin plus egg yolk with linseed oil to give the reduced (for egg) C9/C16 or C16/C18 values.
	red	Animal glue and egg yolk.
56.	red	As above. μRaman showed the presence of massicot in the sample.
	blue	As above. No μRaman was performed in this sample.
57.	red	Animal glue, egg yolk and linseed oil.

58.	red	Egg yolk only. AA ratios data correct, C9/C16 and C16/C18 as expected.
59.	ground	Animal glue and linseed oil (correct AA ratios for animal glue). μRaman indicated also the use of gypsum.
	orange	Egg yolk was detected in both paint samples.
	red	As 58 red above.
60.	red	Egg yolk plus linseed oil.
61.	red	Egg yolk and possibly walnut oil–not in 1:1 ratio as per reference.
62.	red	As above.
63.	white	Egg yolk only.
64.	white	Animal glue, egg yolk and poppy oil – egg/poppy oil not in 1:1 ratio.
65.	red	μFT-IR detected the presence of egg yolk and red lead. μRaman indicated the presence of red lead with cinnabar a: it was found through EDX analysis. GC verified the presence of egg yolk.
66.	white	Animal glue and egg yolk. Even though the C16/C18 ratio is 2.3 indicative for egg yolk, the high value of C9/C16 ratio (1.1) suggests the possible presence of a drying oil not in 1:1 ratio.
67.	red	Egg yolk only.
68.	white	Egg yolk and poppy oil.
	blue	Egg yolk and poppy oil.
	yellow	Walnut oil.
69.	cross-section	Paint: emulsion. Ground: protein.

Table 5.4 Results from icons studied from Zakynthos

Icon	Samples	Pigments				Binding medium									Analytical Techniques				
						Protein				Drying Oil				Emulsion					
		Lead white	Cinnabar	Red lead	Other	Egg yolk	Animal glue	Casein	unidentified	Linseed oil	Walnut oil	Poppy oil	unidentified	Y/N	SEM/EDX	µFT-IR	GC	*Raman*	*Cross-sections*
70	70-grey	x			black (Fe, C)										x		x		
	70-white	x				x	x			x				Y	x		x	x	
71	71-white	x								x				N	x		x		
72	72-red		x	x						x				N	x	x	x		
	72-white	x								x				N	x	x	x		
73	73-pink	x	x			x						x		Y	x	x	x		
	73-light blue	x			smalt	x				x				Y	x		x		
74	74-white	x									x			N	x		x		
	74-red			x							x			N	x		x		
75	75-red		x	x			x			x				N*	x		x		
	75-white	x					x			x				N*	x	x	x		
76	76-red			x						x				N	x	x	x		
	76-rose	x		x								x?		N	x		x		
77	77-red		x	x		x	x			x				Y	x		x		
	77-white	x				x	x			x				Y	x		x		
78	78-yellow				PbO, Fe_2O_3					x				N	x		x		
	78-white	x								x				N	x		x		
79	79-white	x				x	x			x				Y	x	x	x	x	
80	80-grey	x			black (Fe, C)										x				
	80-white	x									x			N	x	x	x		
81	81-white	x								x				N	x	x	x		
82	82-beige	x			Fe_2O_3					x				N	x		x	x	
	82-white	x					x			x				N*	x	x	x	x	

Icon	Samples	Pigments				Binding medium									Analytical Techniques				
						Protein				Drying Oil				Emulsion					
		Lead white	Cinnabar	Red lead	Other	Egg yolk	Animal glue	Casein	unidentified	Linseed oil	Walnut oil	Poppy oil	unidentified	Y/N	SEM/EDX	µFT-IR	GC	*Raman*	*Cross-sections*
83	83-grey	x			black (C)					x				N	x		x		
	83white	x								x				N	x		x		
84	84-red		x	x											x				
	84-white	x				x	x			x				Y	x		x		
85	85-white	x				x	x			x				Y	x	x	x		
86	86-white	x				x				x				Y	x		x		
87	87-red		x												x		x		
	87-white	x				x	x			x				Y	x	x	x		
88	88-red		x			x				x				Y	x	x	x		
	88-white	x								x				N	x		x		
89	89-beige	x			Fe_2O_3		x	x						N	x		x		
	89-white	x								x				N	x		x		
90	90-red		x	x		x?	x			x				Y	x		x		
	90-white	x				x				x				Y	x	x	x		
91	91-red		x	x								x		N	x	x	x		
92	92-white	x				x	x			x				Y	x		x		
93	93-red		x			x	x			x				Y	x	x	x		
	93-white	x				x	x			x				Y	x		x		
94	94-blue				indigo Pb	x	x			x				Y	x		x	x	
	94-light blue	x			indigo	x						x		Y	x		x	x	
	94-red		x			x				x				Y	x	x	x	x	
	94-white	x			$CaCO_3$, $CaSO_4$	x				x				Y	x		x	x	
95	95-blue				azurite, Pb	x								N	x		x		
	95-red		x			x	x					x		Y	x		x		

Icon	Samples	Pigments				Binding medium									Analytical Techniques				
						Protein				Drying Oil				Emulsion					
		Lead white	Cinnabar	Red lead	Other	Egg yolk	Animal glue	Casein	unidentified	Linseed oil	Walnut oil	Poppy oil	unidentified	Y/N	SEM/EDX	µFT-IR	GC	*Raman*	*Cross-sections*
96	96-gesso					x	x							N	x		x		
	96-white	x				x	x			x				Y	x		x		
97	97-red		x	x		x	x			x				Y	x		x		
98	98-white	x								x				N	x	x	x		
	98-grey	x			black (Fe, C)					x				N	x		x		
99	99-white	x								x				N	x		x		
100	100-grey	x			black (C, Ca)	x	x			x				Y	x		x		
	100-gesso				$CaSO_4$										x				
	100-white	x				x	x			x				Y	x	x	x		
101	100-red		x	x						x				N	x		x		
	100-white	x								x				N	x		x		
102	102-red		x			x				x				Y	x	x	x		
	102-white	x								x				N	x		x		
103	103-red		x	x						x				N	x	x	x		
104	104-red		x							x				N	x	x	x		
105	105-white	x								x				N	x	x	x		
106	106-white	x				x	x			x				Y	x	x	x		
	106-red		x				x			x				N*	x		x		
107	107-white	x								x				N	x	x	x		
108	108-red		x	x			x			x				N*	x	x	x		
	108-pink	x	x												x				

Icon	Samples	Pigments				Binding medium									Analytical Techniques				
						Protein				Drying Oil				Emulsion					
		Lead white	Cinnabar	Red lead	Other	Egg yolk	Animal glue	Casein	unidentified	Linseed oil	Walnut oil	Poppy oil	unidentified	Y/N	SEM/EDX	µFT-IR	GC	*Raman*	*Cross-sections*
109	109-white 1	x				x	x			x				Y	x		x		
	109-white 2	x				x	x			x				Y	x	x	x		
110	110-red		x	x						x				N	x	x	x		
	110-white	x								x				N	x		x		
111	111-grey	x			black (C, Ca)					x				N	x	x	x		
	111-beige	x			Fe_2O_3					x				N	x		x		
112	112-white 1	x								x				N	x		x		
	112-white 2	x								x				N	x		x		
113	113-red		x	x		x	x			x				Y	x	x	x		
114	114-white	x								x				N	x	x	x		
	114-green									x				N			x		
115	115-red		x	x						x				N	x		x		
	115-white	x				x	x			x				Y	x		x		
116	116-white	x								x				N	x	x	x		
	116-yellow	x			Fe_2O_3					x				N	x		x		
117	117-white	x								x				N	x		x		
118	118-white	x								x				N	x		x		
119	119-white 1	x								x				N	x		x		
	119-white 2	x				x				x				Y	x		x		
120	120-red		x	x						x				N	x	x	x		
	120-white	x								x				N	x		x		
121	121-red		x			x				x				Y	x	x	x		
	121-white	x				x				x				Y	x		x		

5.1.4.1 Summary of analyses: Icons 70 – 121

70.	grey	The sample was so hydrolysed that no peaks related to AA or FA were detected.
	white	The AA ratios showed that the medium used was egg yolk and animal glue. The FA ratios indicate the presence of a drying oil. The C16/C18 value of 1 indicates the use of stand oil.
71.	white	Linseed oil.
72.	red	μFT-IR suggested the use of a drying oil. The GC analysis
	white	of both samples showed that the oil used was linseed oil.
73.	pink	Emulsion of egg yolk and poppy oil.
	light blue	Emulsion of egg yolk and linseed oil.
74.	white	Walnut oil alone was the medium detected in both
	red	samples.
75.	red	In both samples animal glue and oil were detected. In the
	white	white linseed oil was found, while the white one seems to contain the stand oil with a C16/C18 ratio 0.7.
76.	red	Linseed oil.
	rose	The present sample was highly hydrolysed. No AA were detected. The presence of FA did not help the identification a lot due to the fact that C18 was almost non detectable. Thus, the ratio of C16/C18 with the value of 4.9 (poppy oil) cannot lead to a safe result.
77.	red	The value of G/A and L/A of 0.7 indicate the use of egg yolk and animal glue. The high value of C9/C16 (1) indicates the presence of a drying oil and the C16/C18 of 1.1 suggests the possible presence of modified drying oil.
	white	The AA ratios indicate the use of egg yolk. The presence of OH-P verifies the use of animal glue, while the FA ratios indicate, as above, the use of stand oil.
78.	yellow	The FA ratios indicate the presence of linseed oil.

	white	The FA ratios indicate the presence of stand oil.
79.	white	The GC analysis suggests the use of an emulsion. The AA ratios and distribution indicate the use of egg yolk. The increased value of G/A suggest also the presence of animal glue. Linseed oil seems to have been used as well, from the C9/C16 (1.9) and C16/C18 (1.8) ratios.
80.	white	In this sample no AA were detected. The C16/C18 value of 2.5 is close to the value for walnut oil.
81.	white	Linseed oil.
82.	beige	In the beige sample no AA were detected. The FA ratios suggest the use of linseed oil.
	white	On the contrary, AA were detected in the white sample. The G/A ratio of 1.6 along with the presence of OH-P indicate the use of animal glue. The elevated value of L/A of 1.4 suggest also the presence of egg yolk. The FA ratios indicate the addition of linseed oil not in 1:1 ratio.
83.	grey	Both samples contain drying oil as the medium. The
	white	C16/C18 FA ratio indicates the use of stand oil. The grey sample presented a remarkable high level of C9 possibly due to the extended degree of ageing.
84.	white	The OH-P detection suggests the presence of animal glue. Additionally, the elevated value of L indicates the possible presence of egg yolk. The FA ratios show the use of an emulsion with linseed oil.
85.	white	As above.
86.	white	The AA and FA ratios suggest the presence of an emulsion consisting of egg yolk and stand oil.
87.	red	The red sample was too decayed. Thus no results were obtained.
	white	The samples of this icon were quite hydrolysed. The AA and FA values of the first sample suggest the use of an emulsion from egg yolk and linseed oil. Additionally the

		detection of OH-P recommends the presence of animal glue.
88.	red	Emulsion of egg yolk and stand oil.
	white	Linseed oil.
89.	beige	This sample gave quite interesting results. In the beige
	white	sample, the GC analysis detected casein along with animal glue. On the contrary, the white one contained a drying oil only. In the artists' texts (Chapter 2) the use of different medium layers has been described. i.e. the use of an oily layer and then a proteinaceous layer on top.
90.	red	This analysis of this sample showed the use of animal glue and linseed oil. Egg yolk should be expected but the AA cannot help to identify its presence. However, the value of C9/C16 is representative of an emulsion, thus this could be used as an indication.
	white	At the white sample egg yolk and linseed oil were detected.
91.	red	Poppy oil.
92.	white	The AA ratios indicated the use of egg yolk and animal glue. The L and I did not derivatize as expected. Additionally, the FA ratios suggested the use of linseed oil.
93.	red	Egg yolk, animal glue and linseed oil.
	white	Egg yolk, animal glue and stand oil.
94.	blue	Emulsion of egg yolk and linseed oil. Additionally, the AA ratio of G/A of 2.4 indicates the presence of animal glue. μRaman indicated the use of indigo.
	light blue	Emulsion of egg yolk with poppy oil. μRaman indicated the use of indigo in this sample as well.
	red	Emulsion of egg yolk with linseed oil.
	white	Emulsion of egg yolk with linseed oil. The use of a mixture of chalk ($CaCO_3$) and gypsum ($CaSO_4$).

95.	blue red	The AA and FA values are typical for egg yolk. The value of C9/C16 should be lower for egg yolk, but C9 represents also the degree of ageing, thus various values can be expected. The high value of the G/A ratio suggests the presence of animal glue along with the egg yolk. Also the FA ratio of C16/C18 (3.6) indicates the use of poppy oil in the mixture.
96.	gesso white	Animal glue with egg yolk, possibly as a contamination from the paint layer. Emulsion of egg yolk with linseed oil and animal glue, possibly as a contamination from the ground layer.
97.	red	The AA and FA ratios suggest the possible use of egg yolk and linseed oil. The presence of OH-P and the elevated value of the G/A ratio show the presence of animal glue, possibly from the ground layer.
98.	white grey	The GC analysis of both samples detected the presence of linseed oil.
99.	white	This sample is quite hydrolysed and the interpretation cannot be done with safety. However, the FA values of the C9/C16 and C16/C18 ratios show the use of linseed oil.
100.	grey white	The analytical results of both samples showed the use of egg yolk/linseed oil emulsions. The elevated value of G/A for both cases confirm the use of animal glue. In the case of the white, the C16/C18 ratio suggests the presence of stand oil. μFT-IR detected the use of a drying oil not different from the ones detected so far.
101.	white red	Both samples indicate the use of a drying oil only. In this case, the oil used, as it derived from the FA ratios, is stand oil.
102.	red	The AA ratios suggest the use of egg yolk, while the FA

		ratios (C16/C18: 1.2) of an emulsion with stand oil.
	white	The absence of AA and the ratios of the C9/C16 (1.4) and C16/C18 (1.9) indicate the use of linseed oil alone.
103.	red	The GC results of this sample are representative for linseed oil.
104.	red	As above.
105.	white	The analysis with μFT-IR showed the presence of lead white (3539.2 – 1740.6 – 1408.6 – 1045.4 – 834.8 – 778.1cm^{-1}) and a drying oil (2918.6 – 2850.2 – 1519.1 – 1193.5 – 1098.6 cm^{-1}). Gas chromatography verified the results obtained by FT-IR microscopy. No AA were detected. The FA ratios indicated the use of stand oil.
106.	white	Emulsion of egg yolk and linseed oil. The presence of OH-P confirms the use of animal glue.
	red	The results of this sample show the presence of animal glue (OH-P) and stand oil.
107.	white	Linseed oil.
108.	red	The GC analysis showed the presence of animal glue and linseed oil. However, the high value of C9/C16 (1.4) does not suggest the use of an emulsion, but the use of the drying oil as the paint's medium. Thus the detection of animal glue may come from the layer of the ground preparation.
109.	white1 white2	In both samples the use of egg yolk/linseed oil emulsion was indicated along with the presence of animal glue. The presence of both proteinaceous materials was concluded from the low values of G/A ratio (for animal glue, a higher value should be expected), the high value of L/A and the presence of OH-P.
110.	white red	The binding medium used in this icon was linseed oil. Some AA were also detected by GC as a background noise, possibly as a contamination from nearby layers.

111.	grey beige	The binding medium used in these samples was linseed oil.
112.	white1 white2	The binding medium used in these samples was stand oil.
113.	red	The even AA distribution proposes the use of egg yolk, while the detection of OH-P, the presence of animal glue. The FA ratios suggest the use of an emulsion with linseed oil.
114.	white green	The μFT-IR analysis of the white sample identified the presence of drying oil (3460.9 – 2922 – 2852 – 1180 – 1107.5 – 945.8 cm^{-1}). Gas Chromatography detected stand oil in both samples.
115.	white	The AA ratios lead to egg yolk, while the detection of OH-P suggests the presence of animal glue. The FA ratios indicate the use of stand oil in the mixture.
	red	Stand oil alone.
116.	white	The analysis of the sample with μFT-IR detected the presence of gesso (3409.3 – 1702 – 1623.5 – 1139.6 cm^{-1}), lead white (3538.9 – 1402 - 1044 cm^{-1}) and drying oil (3010 – 2921.7 – 2851.1 – 1632 – 1252.8 – 1172.7 cm^{-1}). These results were verified by Gas Chromatography and detected the use of stand oil.
	yellow	Stand oil.
117.	white	Linseed oil.
118.	white	As above.
119.	white1 white2	The results of this object are quite interesting. Two samples were taken from the two sides. The White1 showed that the medium used was stand oil, while the sample White2 from the other side showed that an egg yolk/ stand oil emulsion was used.
120.	red	μFT-IR detected the use of a drying oil (2929.3 – 2853.2 – 1316.3 – 1246 – 1167.2 – 1054.2. GC indicated the use of

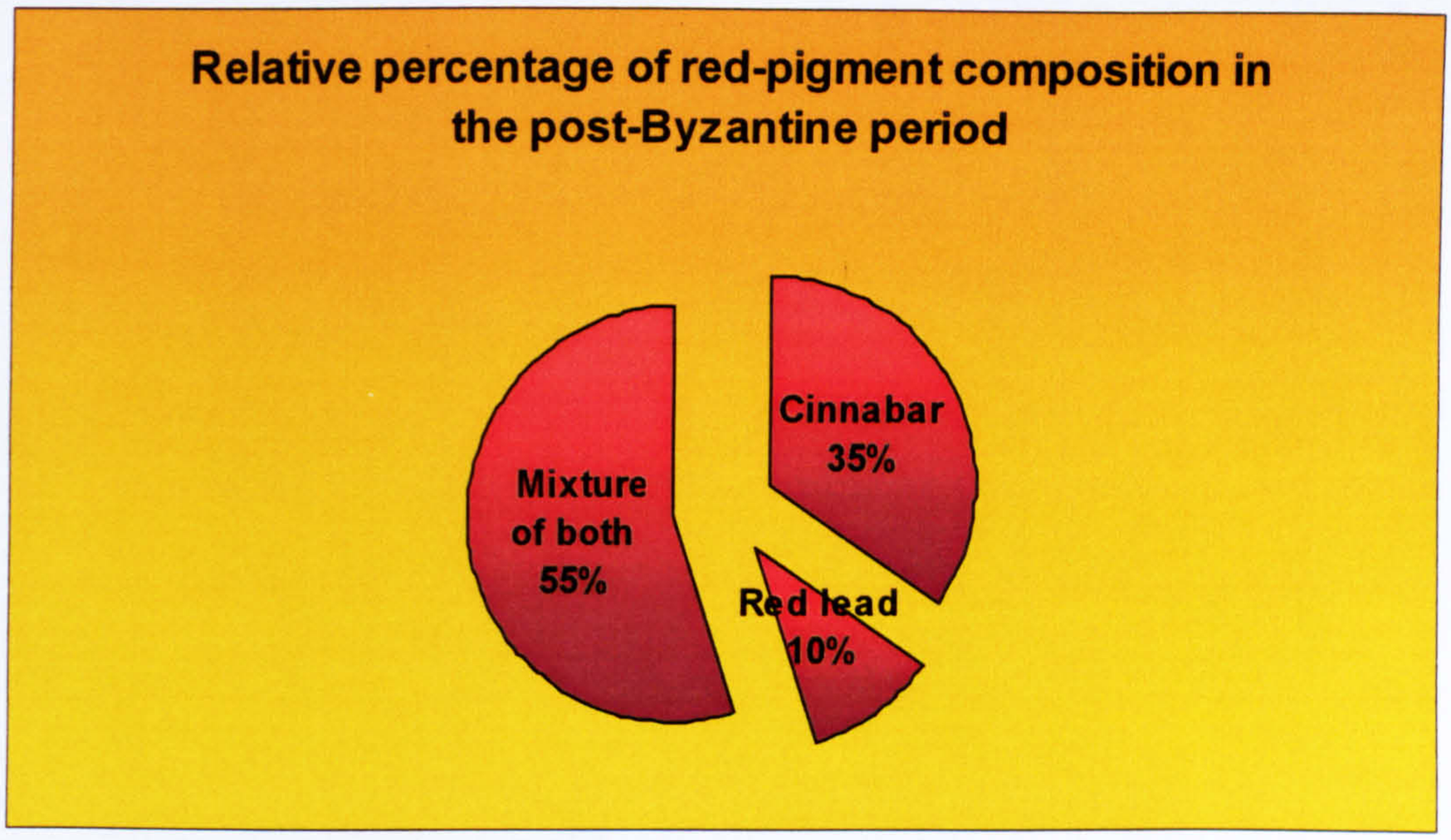
		stand oil.
	white	Linseed oil was detected by Gas Chromatography.
121.	white	Emulsion of egg yolk and linseed oil. The AA ratios along
	red	with the detection of OH-P indicate the presence of animal glue, possibly as a contamination from the ground layer.

5.2 Discussion

As it has already been mentioned (Chapter 4), the choice of the analytical techniques was based on the principle of collection of as much information as possible with non-destructive methods before proceeding to gas chromatography. In several cases the removal of powder samples was not possible, thus the collection of cross-sections and therefore their analyses with micro-chemical techniques was indicated.

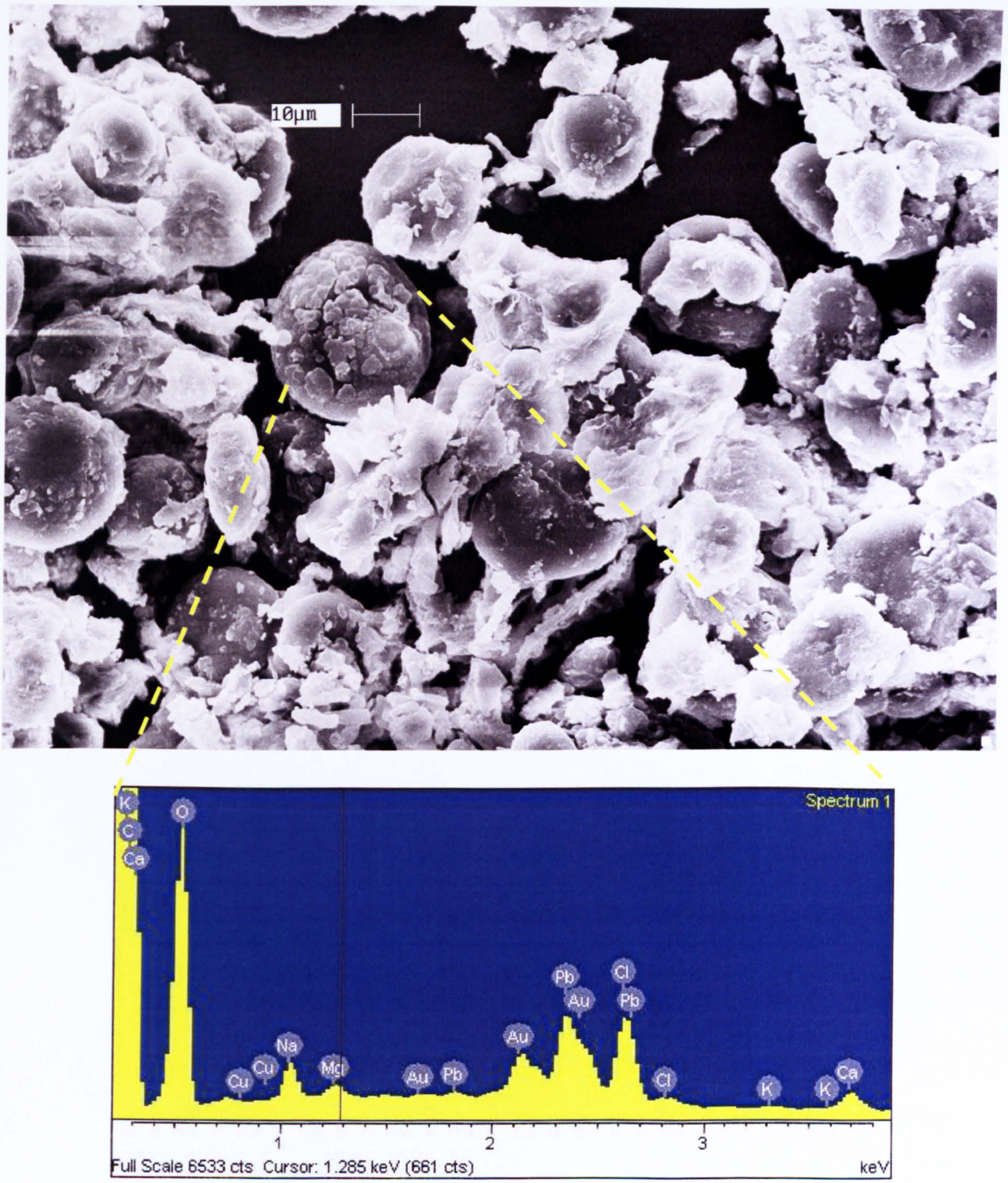
Initially, the determination of the inorganic materials took place. SEM-EDX offered valuable information (Tables 5.1 – 5.4) concerning the elemental composition of the samples, but also concerning the choice of materials used by the artist. As was discussed in Chapter 4, the sampling procedure involved mainly red and white samples. The amount of samples removed from other colours was quite limited (20 out of 201 samples). In all white samples Pb was detected. This element is characteristic for white lead ($Pb(CO_3)_2$. $2Pb(OH)_2$), a typical pigment of the Post-Byzantine period. Hg characteristic for cinnabar (HgS) was detected in quite a few red samples, while an extremely limited number of samples contained red lead (Pb_3O_4) only. However, the biggest percentage of red samples contained large amounts of Pb and Hg, which is indicative for the pigments red lead (minium, Pb_3O_4) and cinnabar (HgS), respectively (Figure 5.1). According to Cennino Cennini (1954) and Dionysios ek Fourna (1906), these pigments were commonly used by the Italian and Greek artists of the 15^{th} to the 18^{th} centuries. In particular, the mixture of cinnabar with minium was widely used, since cinnabar was a very expensive pigment and difficult to find, therefore admixing it with minium would produce a colour of the desirable hue.

Figure 5.1 Composition of red-contained samples



Little information about the different crystals of the samples could be obtained from the SEM images, due to the fact that most images revealed small homogenous lumps. However, sample 66-white gave a very interesting SEM image; small spheres with flakes around them (Figure 5.2). The EDX spectra shown was recorded along the dotted line shown at the bottom of the Figure and represents the average atom percentage of the spheres. The gold signal in the spectrum is from the sample coating. EDX analysis of the spheres showed a significant level of carbon present whilst the data from the flakes indicated lead white to be present.

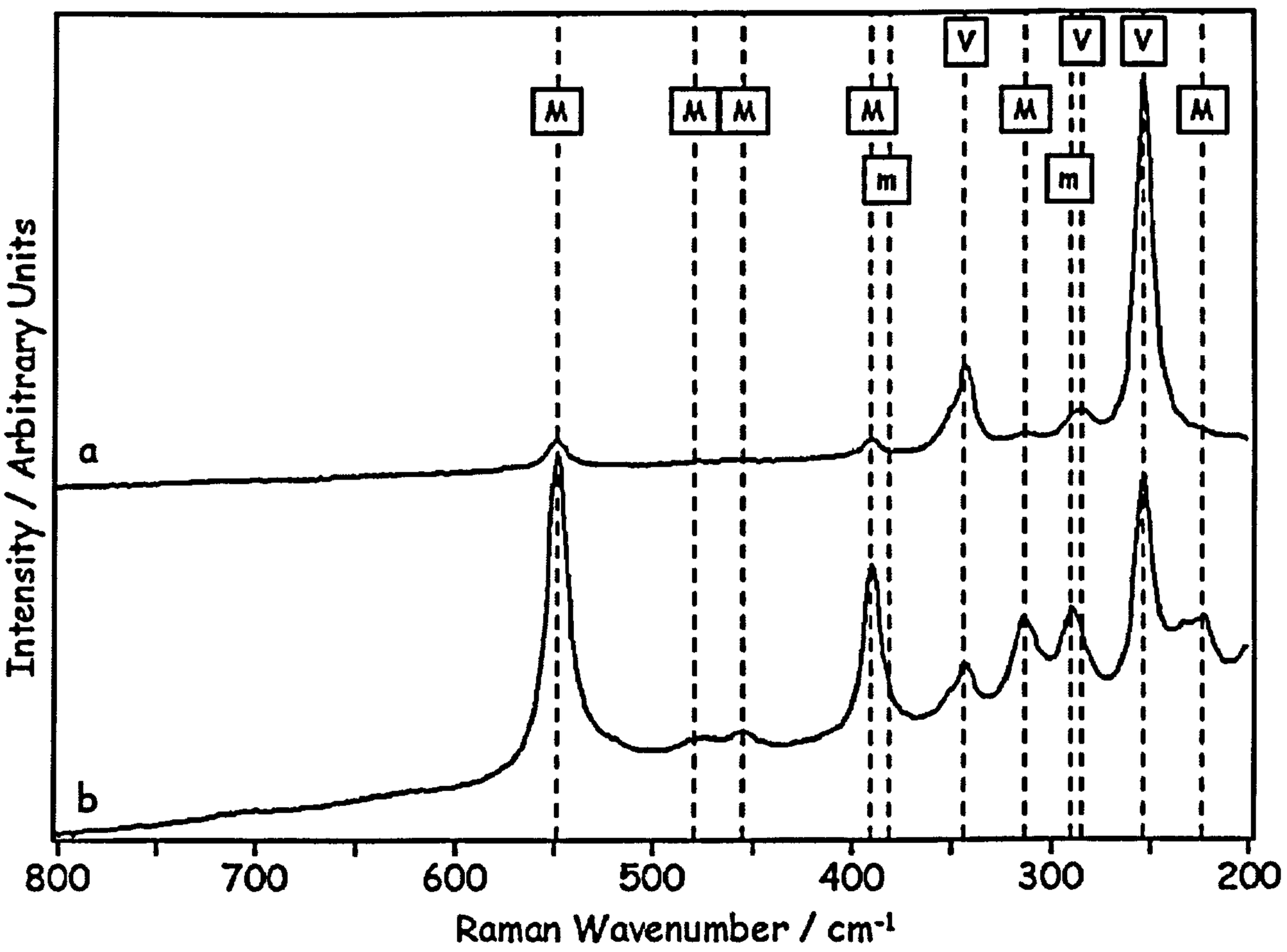
Figure 5.2 SEM image of sample 66-white and EDX analysis of a sphere



The characterisation of pigments was also investigated by means of µFTIR and µRaman spectroscopy. It was interesting to observe the presence of massicot (PbO) in some samples (48white, 53 red, 54 red, 56 red) with µRaman (Figure

5.3). This material may result from the production process of this pigment, since it was generally produced by heating cerussite ($PbCO_3$) or hydrocerussite ($2PbCO_3.Pb(OH)_2$). Under elevated temperatures CO_2 is emitted and the red lead oxide (Pb_3O_4) is produced. Due to inhomogeneous or uncontrolled heating of the pigment mixture, some massicot (PbO) may be formed as a side product. The finding of massicot in some samples illustrates one of the advantages of a multi-method approach; using SEM/EDX alone it would have been difficult to identify this compound.

Figure 5.3 Raman spectra of two red samples. a. 56 red and b. 53 red. Marked Raman bands are: V: Vermilion (HgS), M: Minium (Pb_3O_4) and m: Massicot (PbO).



Concerning the ground layer, Ca was identified. However, the exact type of the ground (either $CaCO_3$ or $CaSO_4.2H_2O$) could not be identified through EDX analysis, in samples containing pigments. This was due to the fact that each time sulphur (indicative for $CaSO_4.2H_2O$) was detected in red samples along with the presence of Hg, it was directly associated with mercury (HgS).

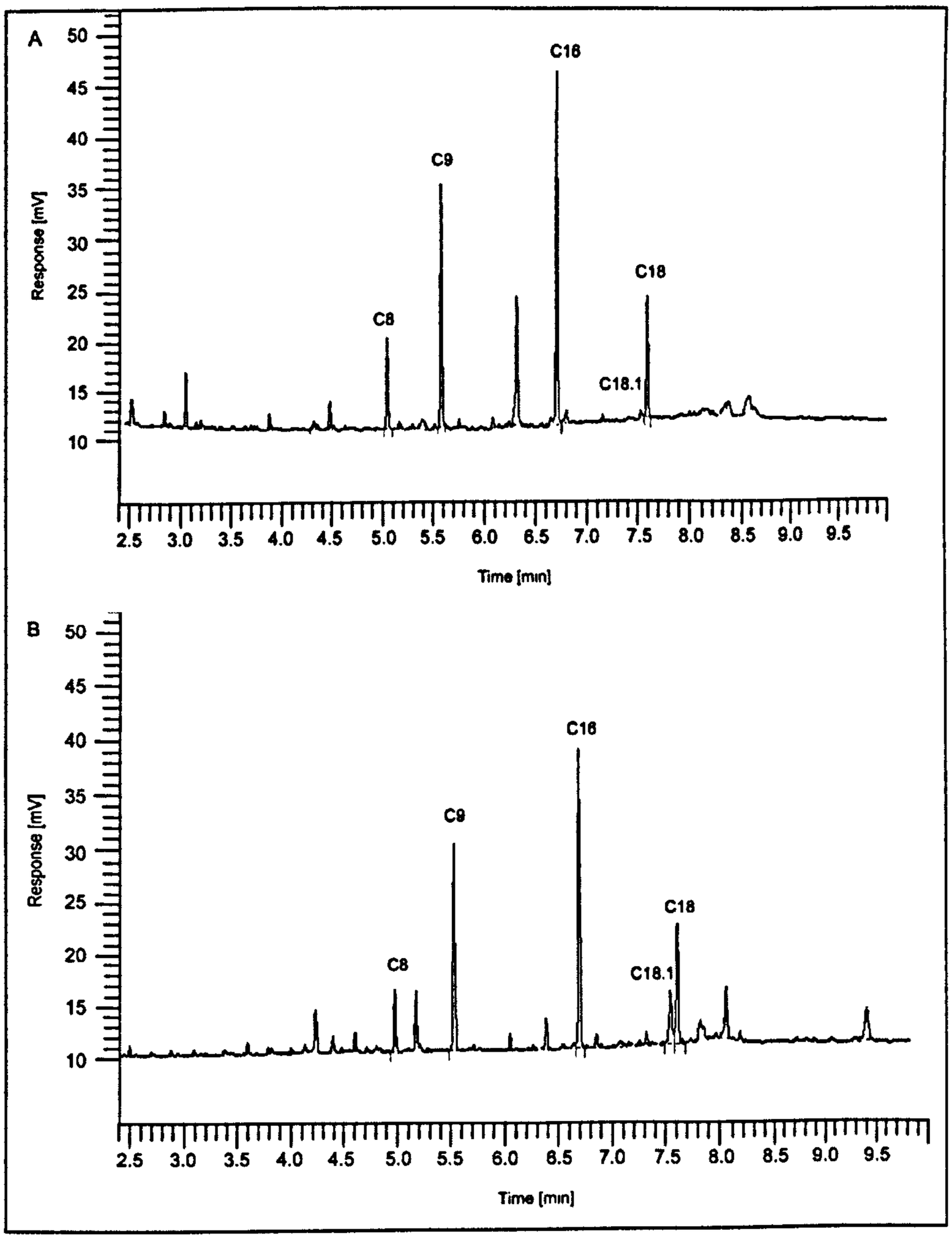
Furthermore, in the white lead containing samples, no sulphur was detected. Only in one sample of ground (100 gesso) EDX detected sulphur, indicative for gypsum ($CaSO_4$. 2 H_2O). Thus, for the characterization of the ground molecular spectroscopy was expected to be of great help. Indeed with μRaman the ground layer in a number of samples (22, 46, 47, 59, 94,100) was identified as containing gypsum ($CaSO_4$.2 H_2O) [1372, 1134, 1006, 669, 618, 492, 413, 317, 210, 181, 122cm^{-1}].

The characterisation of the organic binders was made possible mainly with gas chromatography. From the 121 icons tested, egg yolk was detected in 22, drying oil in 40 and emulsion in 59. The use of the database of reference samples facilitated the interpretation of the results (Table 5.5 – Figure 5.4).

Table 5.5 The fatty acid ratios of the reference drying oil samples and the yellow paint sample from Icon 68

Sample	C9/C16	C18:1/C18	C16/C18
Poppy oil aged	0.4 ± 0.05	1.1 ± 0.1	3.9 ± 0.1
Walnut oil aged	0.7 ± 0.1	0.5 ± 0.05	3.3 ± 0.1
Linseed oil aged	1.0 ± 0.1	1.1 ± 0.1	1.6 ± 0.1
68-Yellow	0.6	0.5	3.0

Figure 5.4 Gas chromatograms of the amino acid and fatty acid derivatives obtained from paint sample 68 yellow (A) and walnut oil reference sample (B)



The most common emulsion used in both Cretan and Ionian Islands' Schools was the egg yolk/linseed oil. From the emulsions detected, 50% was egg yolk/linseed oil, 25% was egg yolk/walnut oil and 25% was egg yolk/poppy oil respectively. Moreover, from the drying oils detected, 84% was a type of linseed oil (raw linseed oil or stand oil), 11% walnut oil and 5% poppy oil. A unique series of results were obtained from the Island of Zakynthos. The use of stand oil was identified from the fatty acid ratios in 18 of the 46 icons studied. Similar fatty acid ratios were not seen in any of the other samples studied in this work.

The icons concerned were by different artists from different periods and they appeared to have no common characteristics, other than Zakynthos, within the limitations of this investigation. According to Mayer (1991) stand oil was widely used by Dutch artists in the 17th century.

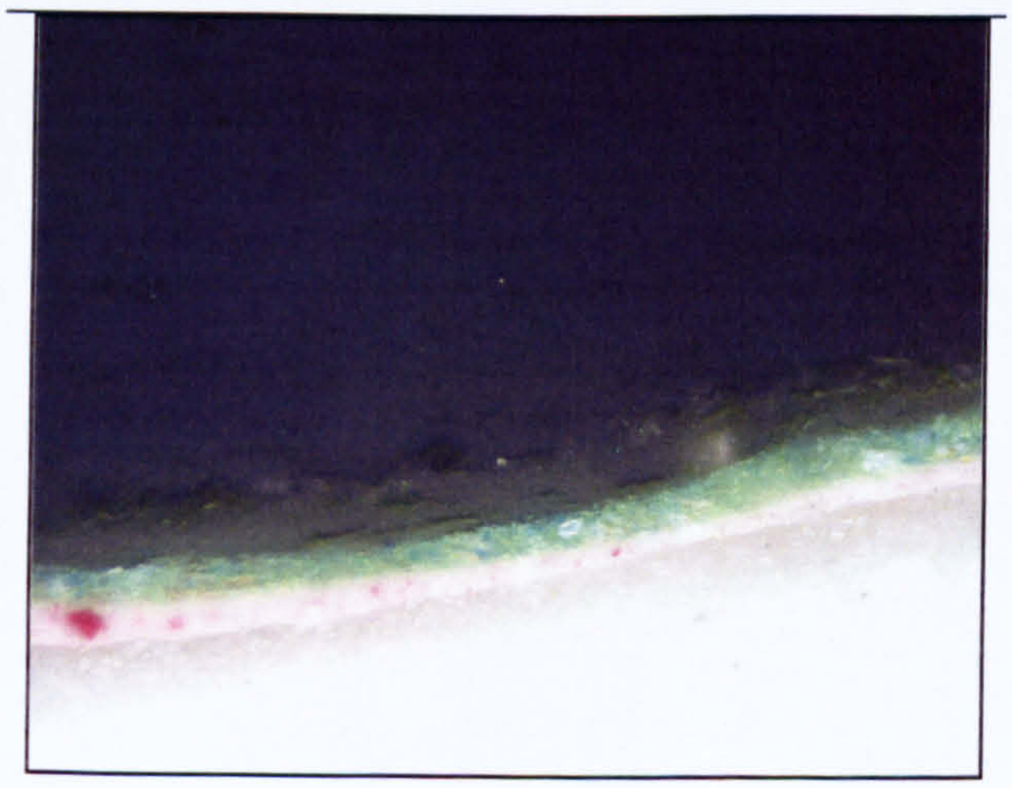
Generally, the use of different use of drying oils for different pigments has been described in artists' manuals (Gettens and Stout, 1966; Mayer, 1991). For example, poppy oil has been suggested as a binder for the white and blue pigments, due to the fact that it does not yellow with ageing. This has been confirmed in this research, since almost all the samples containing poppy oil were of white and blue colour. Likewise, the artists' manuals, especially the ones written by ek Fourna (1906) and Cennini (1954), apart from the use of emulsions, they also refer to the use of drying oil as a layer over the proteinaceous one in order to add plasticity to the painting. This case has also been confirmed in the study of the icon samples by staining the cross sections.

A characteristic example is the 18th century icon of St John Theologos (Figure 5.5) from the homonymous church in Kontogenada, Cephalonia (Figure 5.6).This icon with layers of different composition was found to be unique among twelve studied (Figure 5.7). The others were found to be either emulsions or single components media.

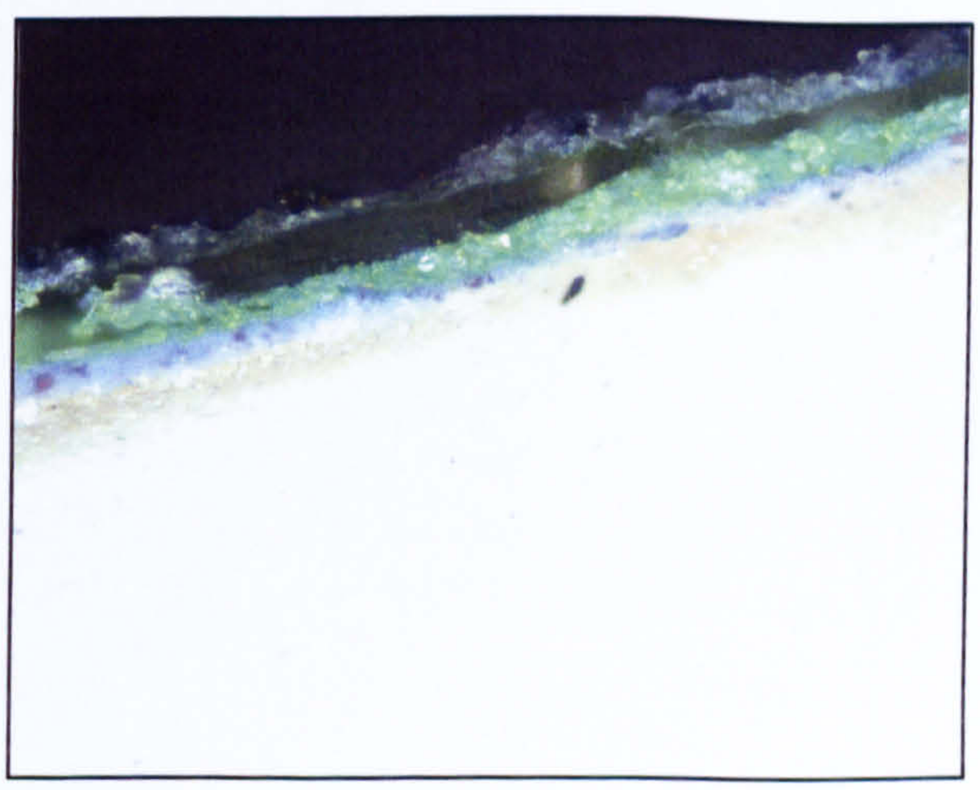
Figure 5.5 The icon of St John Theologos

Figure 5.6 The temple of St John Theologos in Kontogenada, Cephalonia

Figure 5.7 The sample 45 before (a) and after (b) staining. The pink layer was stained by the reagent indicating the presence of protein, while the green layer on top did not stain at all, indicating the use of an oily medium



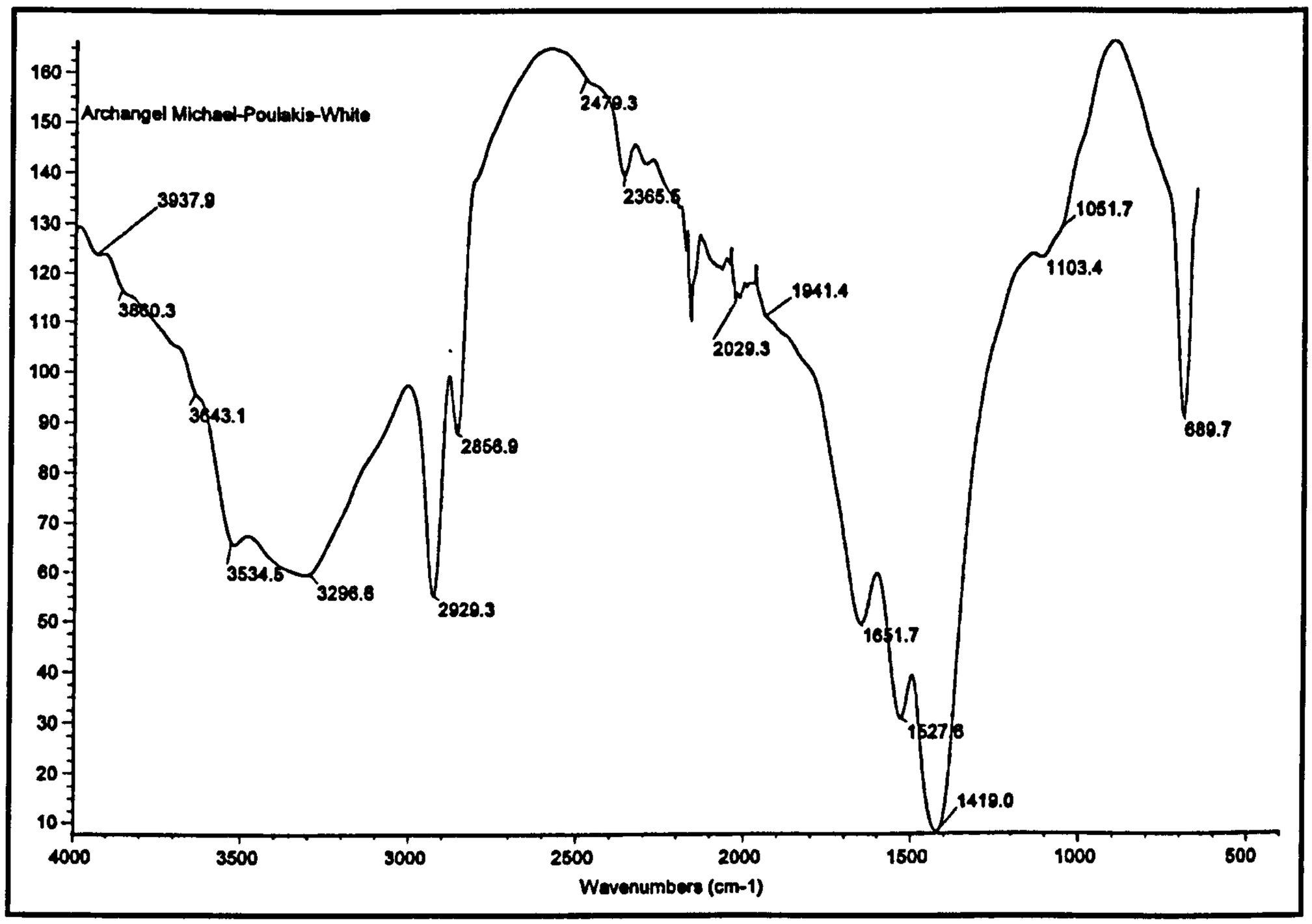
a) Cross-section of sample 45 (magnification 50x)



b) Cross-section of sample 45 after staining with Noir Amide 2 (magnification 50x)

Molecular spectroscopy gave also some information about the organic materials in the samples. Figure 5.8 shows a characteristic example of μFTIR spectrum. Unfortunately, though, these techniques alone without the additional use of gas chromatography cannot provide a safe determination of the binder present. One reason for this is the minute quantity of the organic components present which are usually masked by the absorption bands of inorganic pigments or ground material. Additionally, as it was explained in Chapter 4, the FTIR can detect the presence of oil in a sample, but it cannot identify the type of oil used. Furthermore, in case of emulsions, there is a significant overlapping of the bands arising form the oil with the ones of the egg yolk.

Figure 5.8 FTIR spectrum of 20 white



μFTIR indicated the use of egg tempera-lead white. The main absorption bands that indicate the presence of egg were detected (3296.62cm^{-1}, 1651.7cm^{-1} and 1527.6cm^{-1}). The results were verified by GC.

During the examination and analysis of the 121 icons, there were cases where the characterisation of the binding medium was quite difficult. Dealing with minute samples from icons dating back centuries ago can be quite problematic. Most importantly, the amount of the organic material present in a total sample weighing less than 1mg can be so small that its analysis can be almost impossible. The display or storage conditions for the icons can be critical, for example the exposure to unsuitable environmental conditions such as prolonged exposure to fairly severe humidity, temperature and lighting conditions can lead to chemical change of the binder (Mills and White, 1993). Several of the icons tested fall in this category, since samples were removed from artefacts exhibited in churches by the sea, and museums with unstable microclimates (Figure 5.9 – 5.10).

Figure 5.9 The Church of the Assumption in Cephalonia where the icons are exhibited in a very humid environment

Figure 5.10 One of the galleries of the Post-Byzantine Museum of Zakynthos. The museum is very close to the sea, there is limited environmental monitoring and control and at particular times of the day the sunlight falls directly on the icons

Another critical parameter can be the unrecorded conservation treatments of the icons which may consequently lead to difficulties with the interpretation of results. Even though the sampling procedure was made with extreme care in order to avoid such circumstances, in the case of icons tested from Cephalonia, there was a danger of removing sample from restored areas. This was due to the fact that in 1953 a big earthquake destroyed all the records of the church; therefore information concerning previous treatments was not available.

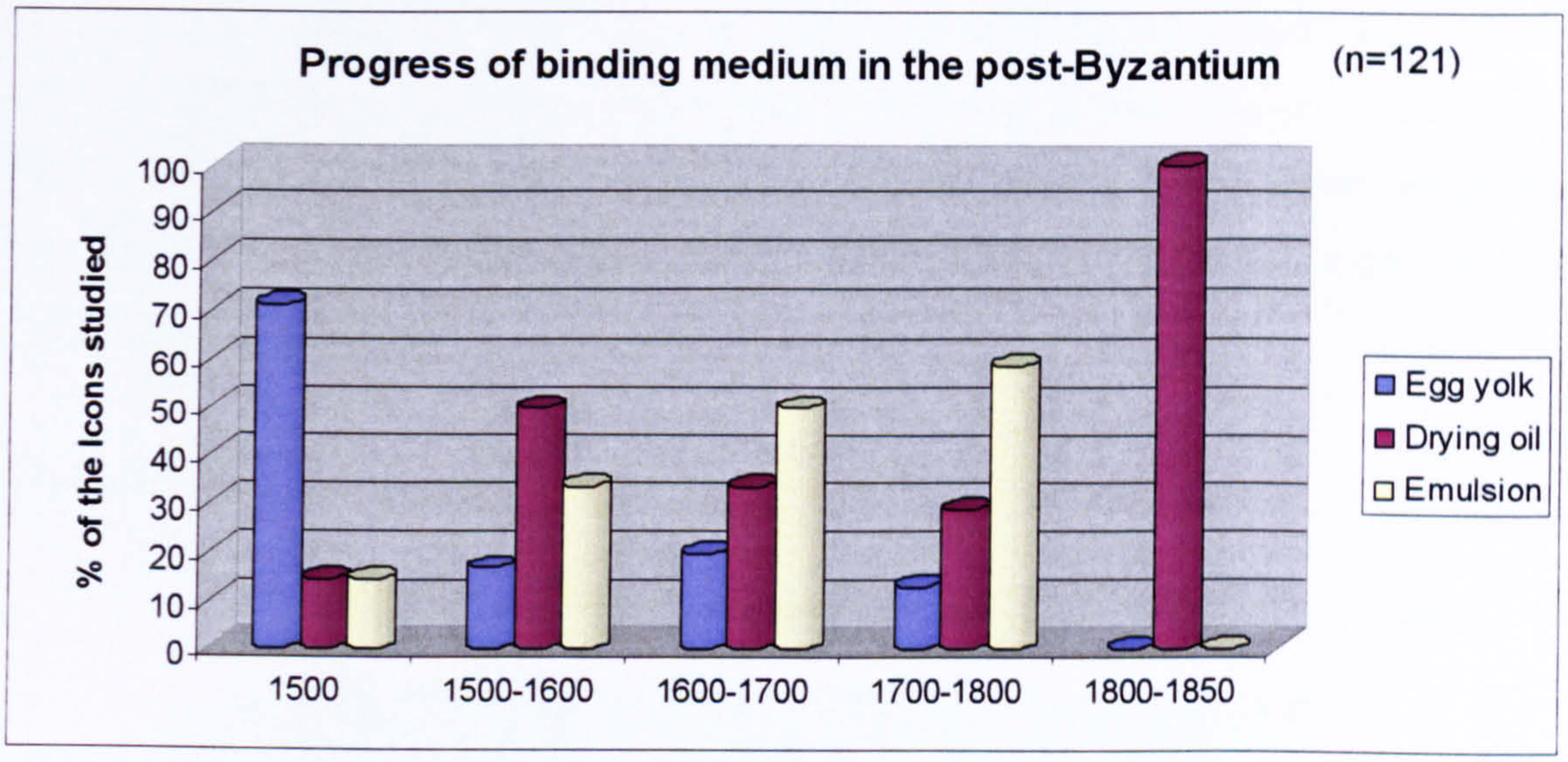
Finally, the analyses were further complicated by the presence of ground layer in some samples which contributed to the amino acid levels. In more than 34% of the total samples studied animal glue was detected. Some painters used to paint extremely thin layers making the collection of the powdered paint samples without some material from the ground layer almost inevitable. The determination of the species present was therefore derived from a careful

consideration of the ratios of the amino acids, the presence of hydroxyproline and the fatty acid ratios C9/C16 and C16/C18.

The characterisation of the components present in the icon samples gave valuable information concerning the artists' materials and techniques of the periods studied, as well as the changes that occurred in them. The interpretation of the results from the amount of samples and icons tested were quite difficult. This was a result of the inhomogeneous sample collection. Unfortunately, when dealing with real artefacts, it is not always possible to collect the desired samples for research. The artefacts determine the sampling procedure and not the other way around. It would be ideal to collect red and white samples of more than 1mg from each icon along with cross-sections for each icon. However, this was not at all possible, since icons do not always have red and white areas and cross-sections cannot be removed unless there are damaged areas in the icons. Additionally, the amount of samples taken depends on the decision of the Ministry of Culture. For example, the author had applied for permission to collect samples from four icons of Theodoros Poulakis exhibited at the Museum of Byzantine Culture in Thessaloniki. The permission was given but they allowed the collection of three samples from the four icons. Thus in order to draw conclusions from the samples obtained, several combinations were tested and performed. Since the introduction of different binding media to the egg tempera technique was the principal subject of this research, the results were analysed with this objective in mind. Therefore, if two samples were tested from an icon and the analysis proved that in the one sample egg yolk was detected, while in the other one emulsion was used, then this icon was recorded for the latest technique, which is the emulsion. Similarly, that was the case for the samples containing drying oils. Furthermore, a comparison of the media used by each artist separately was not among the aims of this research since the icons analysed from each one was used to give a representative example of a specific period and not of the artist himself. This would be more possible if the research had focussed on a number of artists and their work.

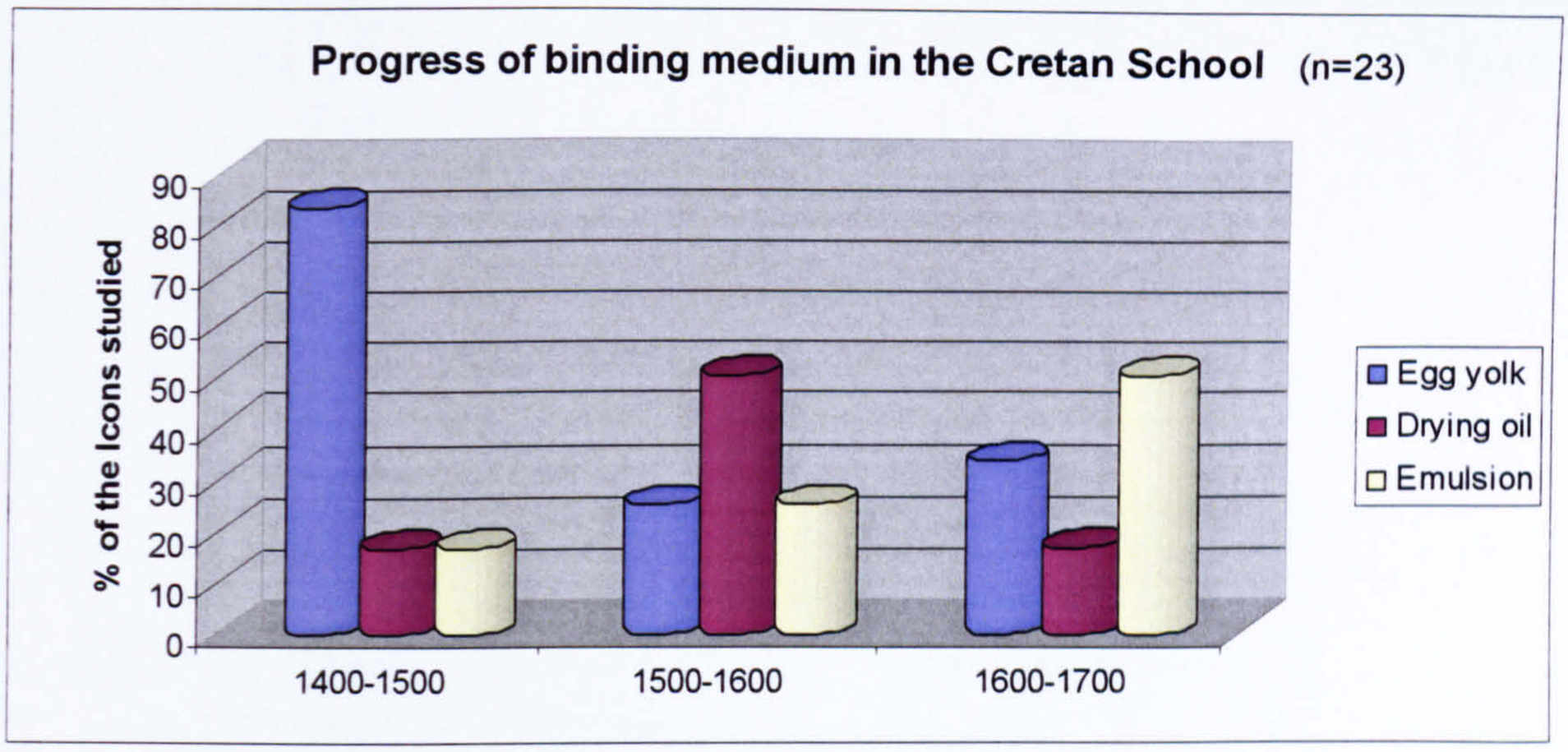
Figure 5.11 illustrates the overall progress of changes in the binding medium in the post-Byzantine era. It is obvious that at the 15th century 70% of the binder used was egg yolk, while with the beginning of the post-Byzantine period the use of egg/oil emulsion and drying oil was introduced. In the 16th century there seems to be a rise in the use of drying oils. As it was mentioned above, artists' manuals (Cennini, 1954; ek Fourna, 1906) also describe the use of oily layer. This is reinforced by the fact that in many of the icons analysed along with the presence of oil, samples containing egg yolk were also detected. From the 17th century a rise in the use of emulsions can be observed, which lasts until the 18th century where the use of drying oils prevails.

Figure 5.11



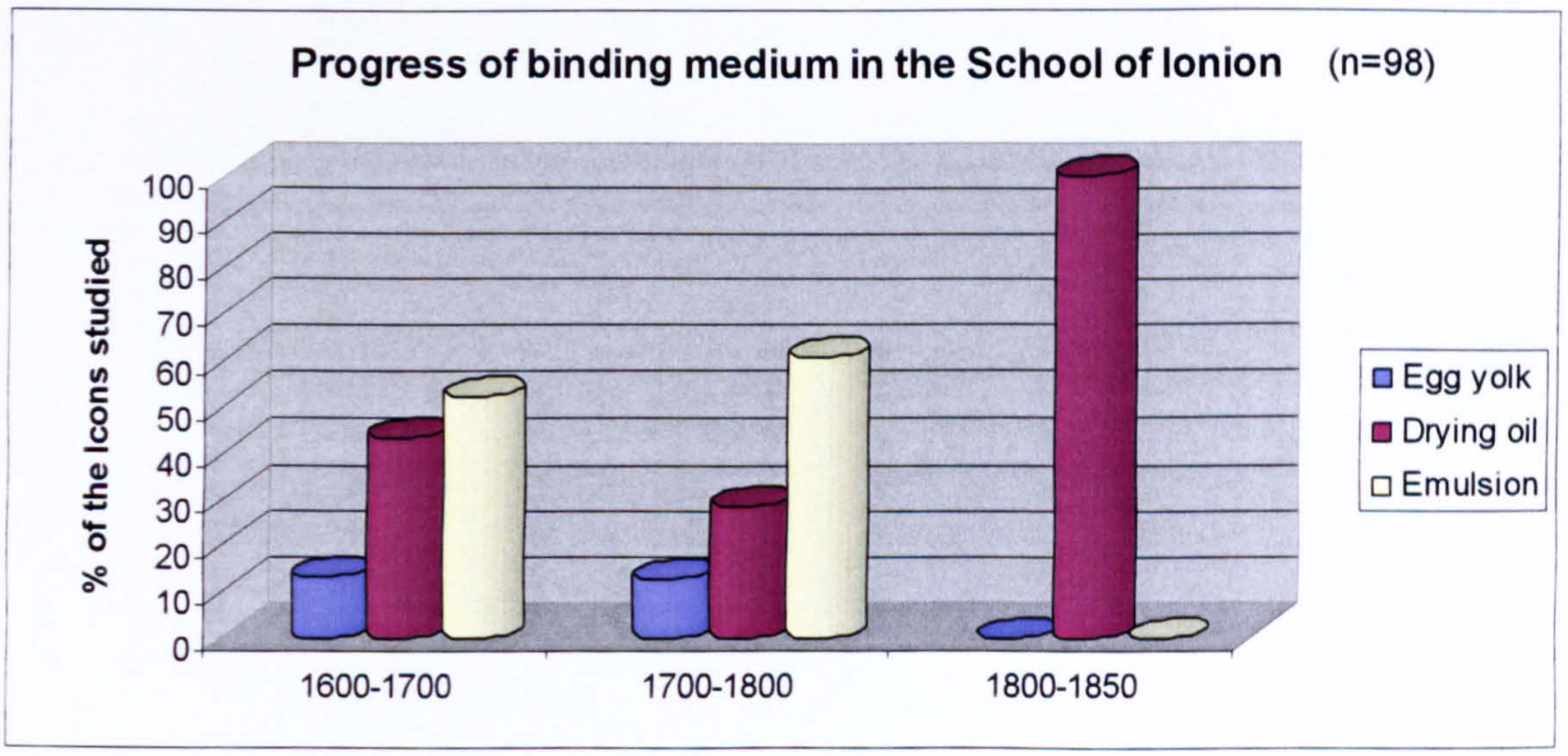
Breaking down these results into more detailed charts divided by Schools (Cretan School and School of Ionion), more information can be obtained about the local progress of the binding media. In Figure 5.12 it is obvious that the artists before the 16th century used mainly egg yolk as the paint's binding medium. In the 16th century there was a rise in the use of oil, while towards the end of the Cretan School in the 17th century, the use of emulsion had been established.

Figure 5.12



At the same time (17th century), the islands of Ionion had already adopted the use of emulsion along with an extended use of drying oils (Figure 5.13). During the 18th century a fall in the use of drying oils and a rise in the use of emulsions has been observed and during the 19th century drying oils seem to be the main binding medium used.

Figure 5.13



A secondary set of outcomes concerning the trends in the islands can be drawn by sub-categorising the chart of the Ionian School. Figure 5.14 shows that

Cephalonia followed a gradual transition from emulsion to oil, while the egg yolk was used until the 18th century. On the contrary, on the island of Zakynthos (Figure 5.2.15), the use of egg yolk was rejected as early as the 17th century and emulsions and drying oils were adopted.

Figure 5.14

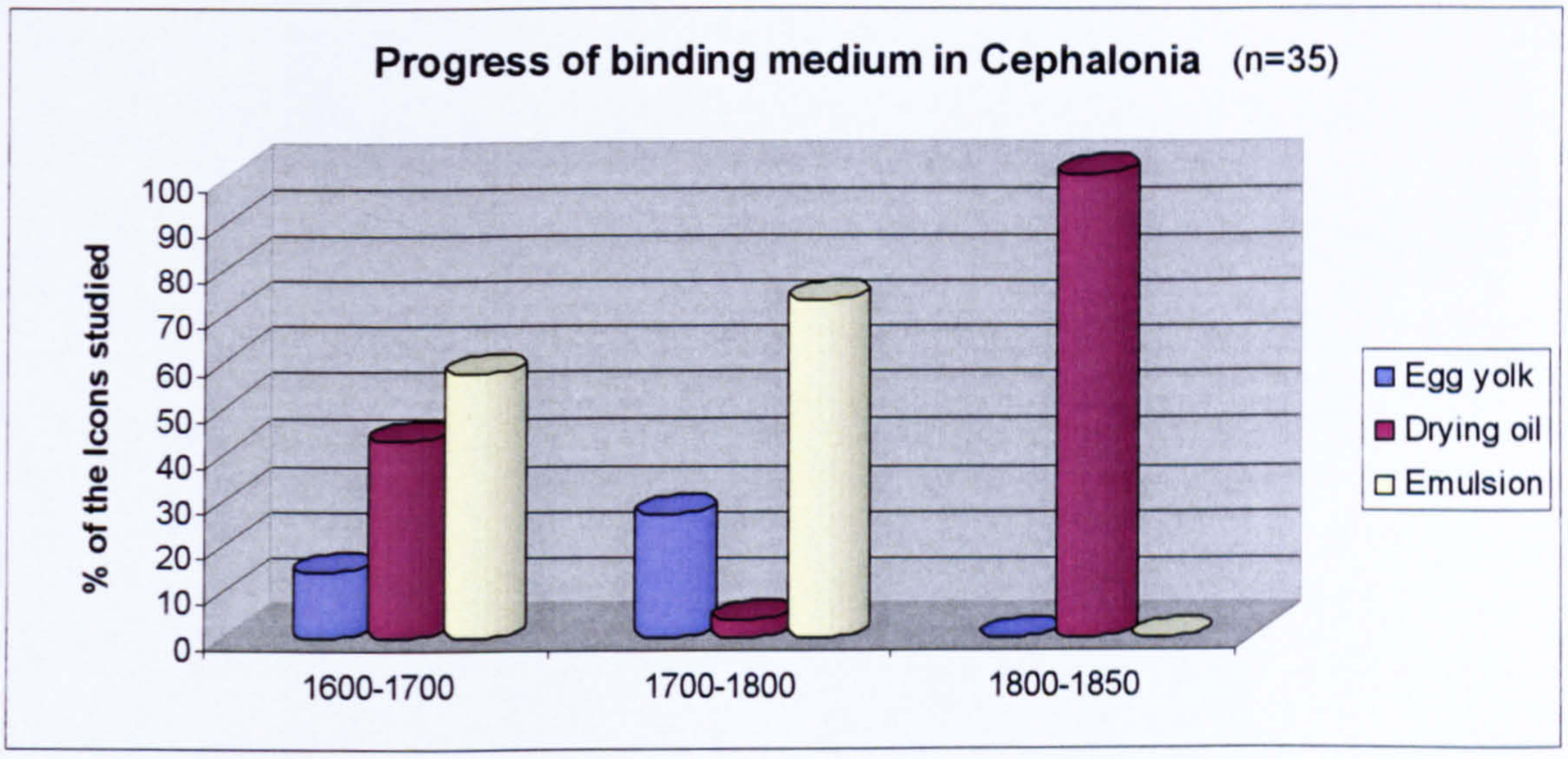
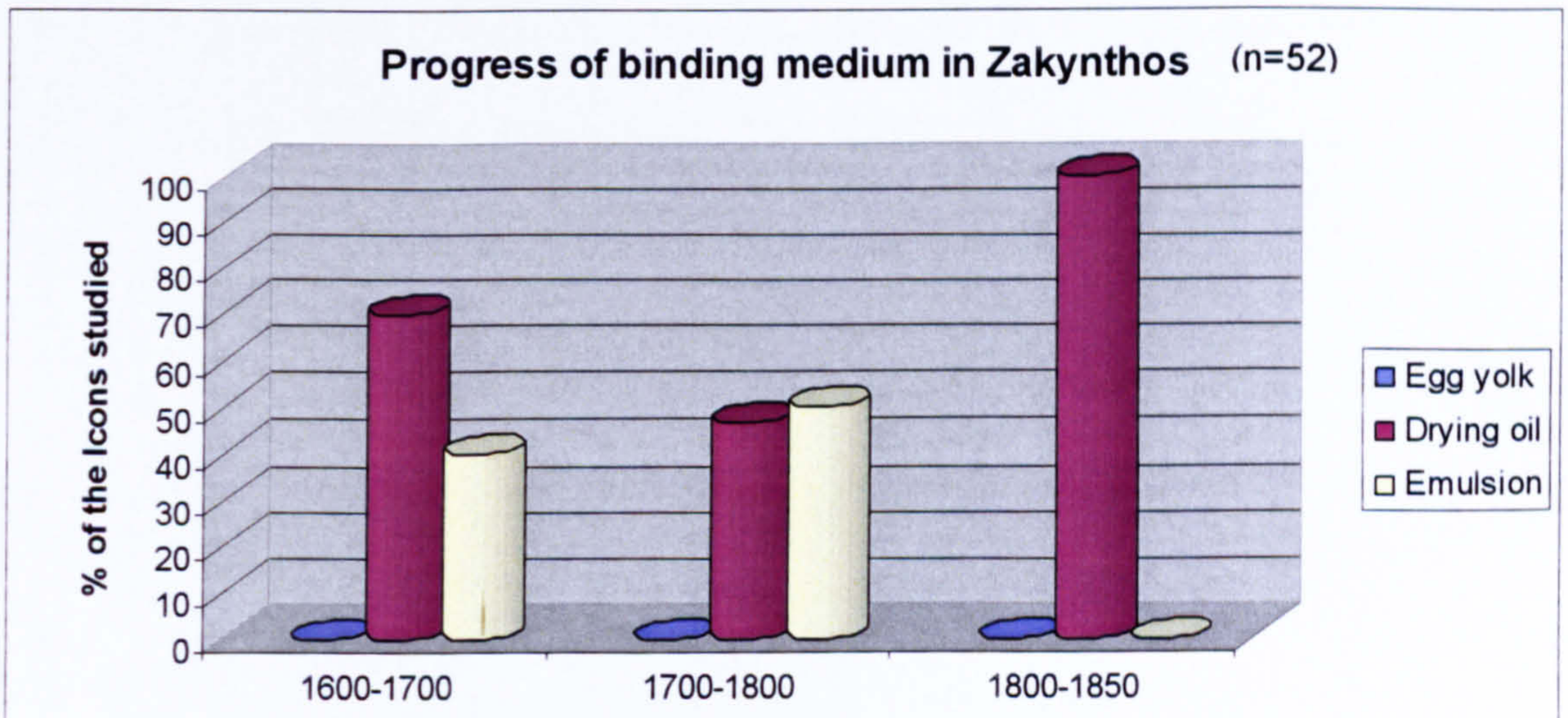


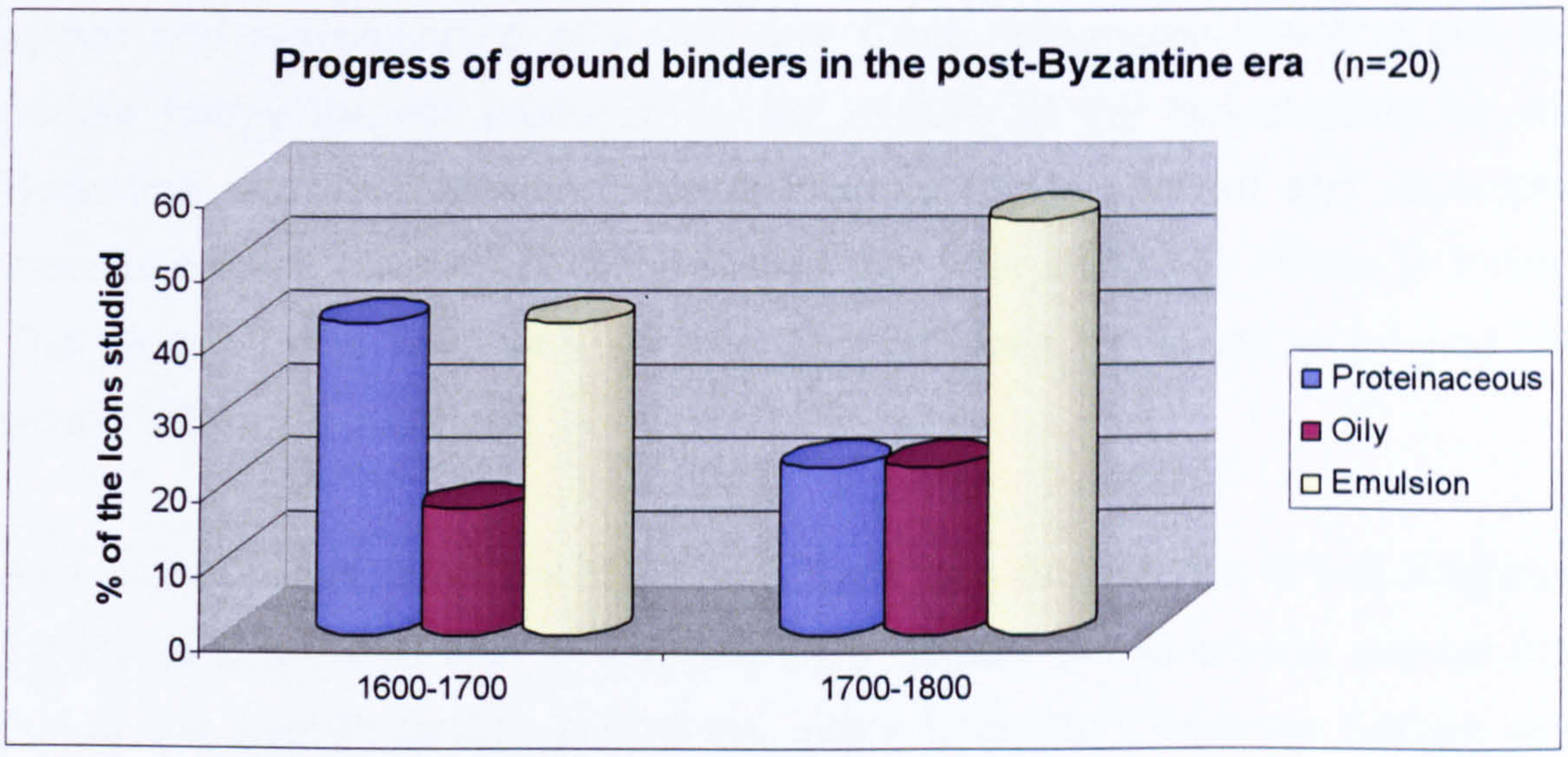
Figure 5.15



Along with the identification of paint's binding media, a small number of ground samples (20) were also analysed. Since the number is very small, only indicative outcomes can be drawn. Figure 5.16 illustrates the use of both

proteinaceous and emulsion binders for the production of the ground layers during the 17th century. The 18th century artists seem to have adopted the use of emulsion. In both centuries a small percentage of drying oils were used as the binder. However, it can be concluded from this chart that even in the ground layer the use of oil was adopted gradually, indicating once more the introduction of Western characteristics in the construction technique of the icon painting.

Figure 5.16



CHAPTER 6
CONCLUSION

6.1. Conclusions

Trying to find answers to the questions raised at the beginning of this research, the author had to set up for a journey at the route of history: Constantinople, Crete, Ionian islands. The landscape itself was indicative of the findings that would follow: Constantinople, the capital of Byzantium, the end of a majestic epoch and the beginning of a new era. Crete, imperious; the land and the people betray the stir produced by the mixture of the two civilizations: the Byzantine and the Venetian. The feeling that things evolved and developed there is evident. As evident is the feeling that everything has settled in Ionion. The landscape is calm and serene. The changes have happened and the people enjoy the outcomes.

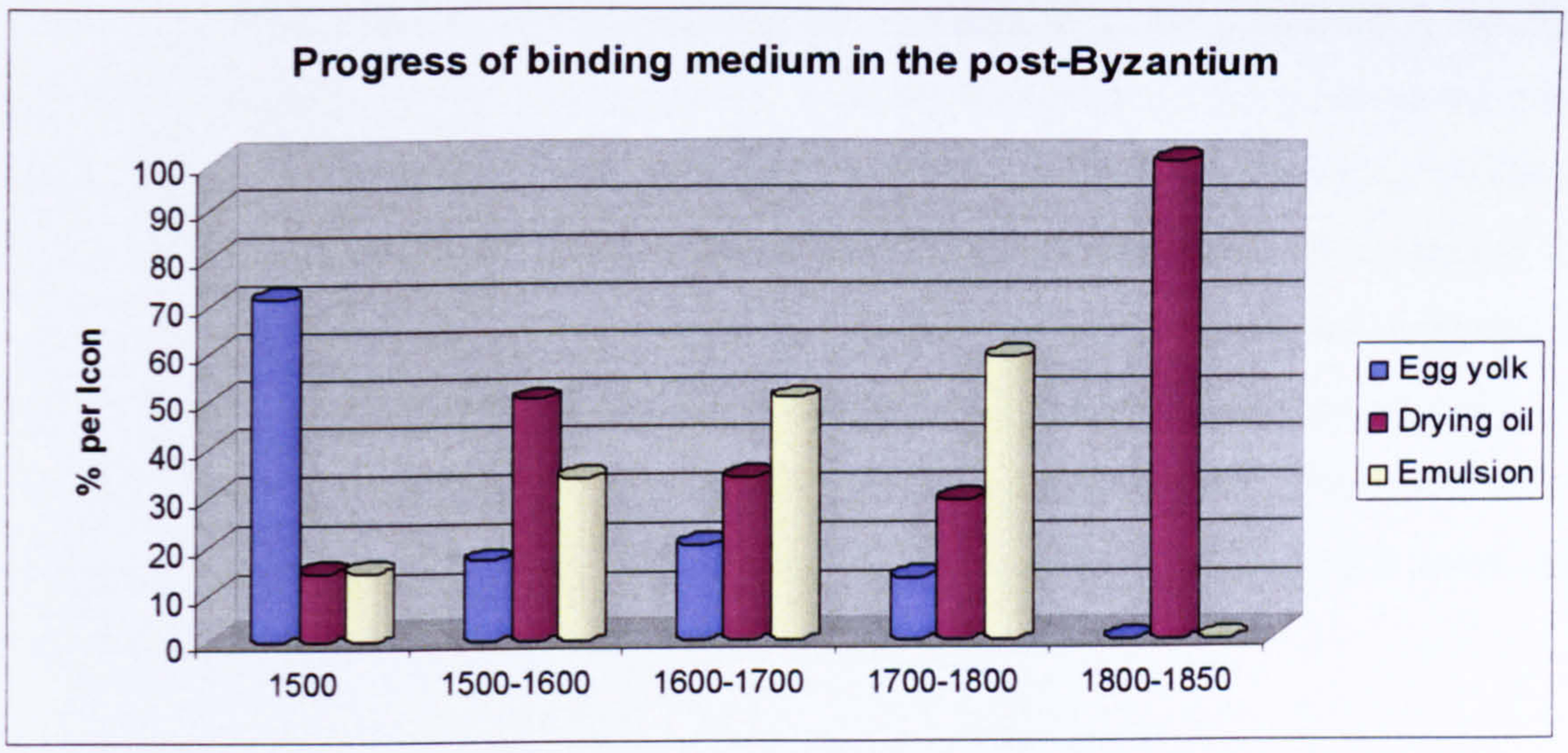
As history reports, after the fall of the Byzantine Empire, the artists migrated initially to Crete and later to the islands of Ionion. Art historians proved that during the post-Byzantine period the interactions with Western Europe and especially with Venice brought radical changes to the painting characteristics of the time; the style gradually changed from religious to secular and from panel painting to oil painting on canvas. However, there was no scientific evidence on how and to what extent those influences affected the techniques and the materials used by the artists.

The aim of this project was to provide information on the nature of the binding media used by the post-Byzantine painters from Crete and the islands of Ionion and to study any possible changes, due to Western influences. The change of the painting characteristics states the extent of those influences and scientific analysis comes to resynthesize the palette of the artist. This was made to produce a more complete idea of this transitional period from which the artists proceeded to secular oil painting.

Scientific analyses provided important information concerning the changes in the paint binding media. The technological background, which was so far available only from artists' handbooks, was now confirmed through analytical science. The research followed the route of the post-Byzantine art and the work of representative artists of the period and whose work also contributed to the evolution of the later pictorial art. A multi-method approach was set up for the collection of as much information as possible from each paint sample.

The current research showed that the cultural exchanges between Western Europe and Post-Byzantium affected deeply the art of the icon painting as a whole. Not only the painting characteristics changed, but the materials changed as well. The artists freed themselves from the strict rules of the Byzantine haghiography and they tried new techniques and new materials coming from Western Europe which had just came out of the Renaissance. The style changed, the forms became more vivid and plastic and the overall synthesis became more human. In the Cretan School of icon painting the use of egg yolk demished during consequent centuries and has been replaced by emulsions (Chapter 4, p.25). It has also been established by this research that in the Ionian School of icon painting, drying oils came to dominate the binding media during consequent centuries (Chapter 4, pp.25-26). Egg yolk ceased being the only material for the mixing of the pigments and drying oils, either as an additive layer or mixed with the egg came to replace and go beyond the egg tempera technique. Greece had experienced radical changes; Byzantium had fallen, the biggest part of the Greek state was under the Ottoman occupation where the Eastern ideology was instituted. On the other hand, the islands of Crete and of Ionion were under the Venetian Rule. It was inevitable for art to record those changes (Figure 6.1).

Figure 6.1



6.2. Recommendations

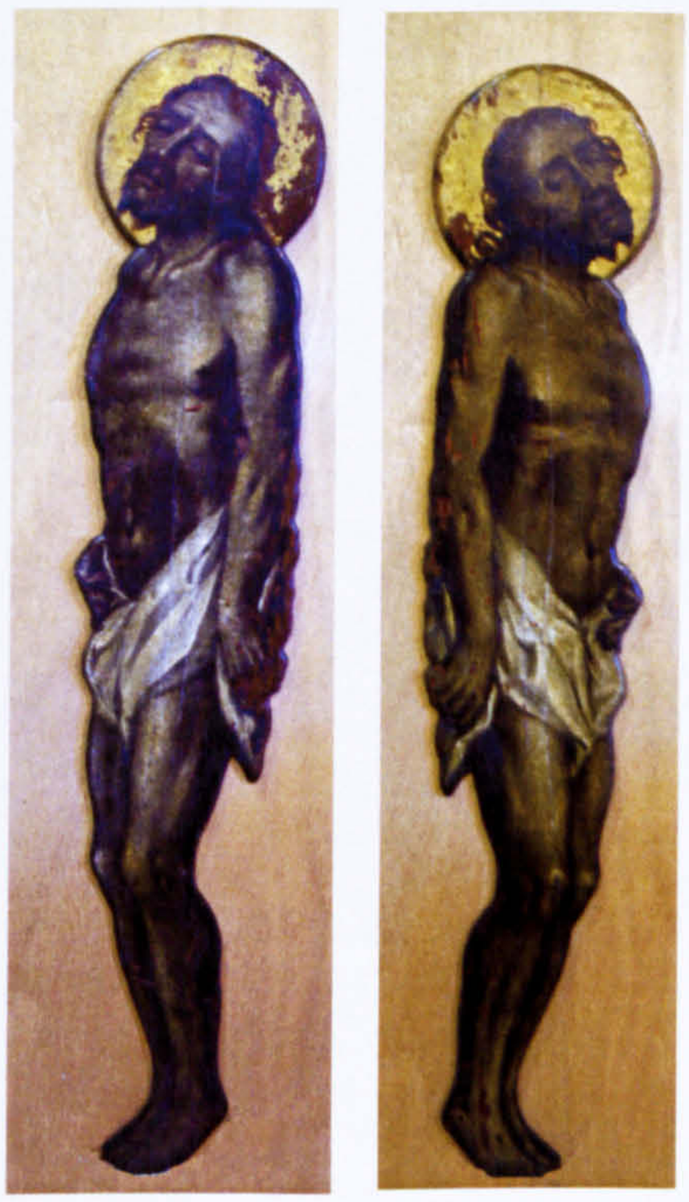
Research never ends as one question leads to another. This specific project opened a pathway by investigating the post-Byzantine icon painting technique. Further work towards this direction could enlighten more this period and the characterisation of the organic materials.

At first, more analytical techniques could be explored in order to provide additional information. Already, a research group based on this project was created at De Montfort University, in order to work on pyrolysis-gas chromatography. A series of tests were made on reference samples (Appendix V) and the characterisation of the nature of the materials tested seems to produce positive results. Extended work on this instrumental technique can prove valuable for the collection of information concerning also the use of additional materials in the organic binders with nilpotent sample preparation. The author is also collaborating with the Foundation of Research and Technology (Forth-HELLAS) in Crete on the use of Nuclear Magnetic Resonance (NMR) on binding media characterisation. This investigation is at the very early stages of the reference sample collection. Furthermore, DNA analysis might provide valuable information on the characterisation of the organic materials, as well as their provenance.

For the progress of this research a set of reference sample was created, based on the materials described by the artists' handbooks. This database could be extended to more materials, covering a wider range of techniques used before and after post-Byzantium and to other layers such as the ground and varnish layer as well. Thus, information about the whole stratigraphy of a typical icon could be obtained. Along with this, the ageing protocols could be extended in order to fully understand the ageing mechanisms of the painting materials. This is among the aims of the Laboratory of Physicochemical Research of the National Gallery of Greece where the author holds a permanent post as a researcher.

Further work could be focused on specific artists or themes. By focusing on specific artists, such as Panayiotis Doxaras and El Greco, important information about several sides of the Cretan School and the School of Ionion could be collected. Likewise, interesting information could be obtained by focusing on specific themes. In Zakynthos, for example, a very popular theme is the depiction of the dead body of Jesus Christ on a double-sided wooden carved panel (Figure 6.2). Some questions which might be asked are: how was this type of painting evolved through the centuries and what was the progress of the binders used.

Figure 6.2 The Lamb, end of 18th c. – beginning of 19th c., Byzantine & Post-Byzantine Museum



Another important area is the preservation of those icons examined. The author is in contact with the sources of the material investigated, in order to help them conserve and exhibit the icons better.

Finally, investigation on the trading, transport and distribution of painting materials through Greece and Western Europe during the post-Byzantine times could prove important for the understanding of the choice of materials.

Post-Byzantium icon painting proved to be an unlimited area of research, where art and technology meet to form history.

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APPENDICES

A/A	NAME	Period	Place of Birth	PLACE OF WORK	CHARACTERISTICS	OTHER
1.	Aggelos Akotantos	1421-1492	Handakas	Handakas & Constantinople	Continues the Constantinople prototypes. Reminds the 1st phase of Cretan School	The older and maybe the most important Cretan painter
2.	Ritzos Andreas	1421-1492	Handakas	Handakas	Uses both Greek and Italian elements	
3.	Tzafouris Nikolaos	Died in 1501	Crete	Crete	Strong influences of the painting characteristics of the 14th c. Italy	
4.	Pavias Andreas	~1504	Crete	Crete	He paints both ala maniera greca (Byzantine prototypes) and ala maniera italiana (later-gothic prototypes)	
5.	Pitzamanos Aggelos	1467- ;	Crete	Crete and Italy	He followed his master's prototypes	Student of A. Pavia
6.	Demetrios	15th –16th c.	Crete	Crete		A great icon painter
7.	Damaskinos Michael	1530/35-1592	Crete	Crete (until1574 and 1584-1590), Venice (1574-1584), and possibly Corfu	Creative absorbance of the contemporary Italian characteristics	
8.	Markos Sterlitzas Mpathas or Vathas	Died in 1578	Crete	Corfu – Venice (1587)	Combines the work of Klontza and Damaskinos	

A/A	NAME	Period	Place of Birth	PLACE OF WORK	CHARACTERISTICS	OTHER
9.	Vathas Thomas (Known only from literature sources)	Died in 1599	Crete	Corfu – Venice	Combines the work of Klontza and Damaskinos.	He left all the under drawings of both Byzantine and Italian styles to Emmanuel Tzanfournari. (1599)
10.	Silvestros Theocharis	16th c.	Handakas – Crete		He mainly follows the prototypes of the later 16th c, influenced more or less by the current Italian art.	Monk and parish priest. Not a very good painter.
11.	Klontzas Georgios	1540-1608	Handakas – Crete	Crete	Many manierist elements – contemporary itslisn tradition. Follows an independent way from the other painters.	Very important master
12.	Emmanuel Lampardos (two painters with the same name)	1587-1631	Handakas – Crete		Returns to models of 15th cent. Quite strict character. In his mature work, there are many Italian characteristics	Son of the priest Nikolaos and the most important of the two
13.	Tzanfournaris Emmanuel	1570/75-1631	Corfu	Corfu – Venice (1599-1631)	Did not absorb characteristics of traditional and Italian art. Remained an eclectic artist, imitating either the Cretan artists of the 15th century, Damaskenos or pure western prototypes.	Student of Thomas Mpathas. Inherited all the under drawings of both Byzantine and Italian styles.

A/A	NAME	Period	Place of Birth	PLACE OF WORK	CHARACTERISTICS	OTHER
14.	Kortezas Georgios	A' half of 17th c.	Handakas – Crete	Corfu – Venice?	Influenced of early Cretan painting with some western influences from Italian works of the 16th c.	Excellent ability to small sized icons.
15.	Emmanuel Tzanes or Bounialis	1610 – 1690	Rethymno – Crete	Crete (until 1646) – Corfu (1648-1654) – Venice (1658-1690) 1658 –1690 Italian period.	Several prototypes, ie. Renaissance	Priest. Use of small white parallel strokes on the flesh – insists on the decorative details– dull colours –usually paints faces with bronze/green colours.
16.	Tzanes Konstantinos	1655 – 1682/85	Rethymno – Crete	Rethymno – Venice (1655-1682/85)	Eclectic artist who other times would paint in a pure Italian style and other times following the orthodox tradition. Other times combining the Italian and Flemish elements and other times the Paleologian style.	The younger brother of Emm. Tzanes. His capability to absorb western influences, together with the sweetish style of his figures created a style that the Catholics liked very much. Big number of icons saved in western churches.
17.	Elias Moskos	17th c.	Rethymno – Crete	Crete – Zakynthos – Corfu	Western standards without leaving the old tradition.	Great ability
18.	Leo Moskos	17th c.	Rethymno – Crete	Crete – Zakynthos – Venice –Cephalonia	Imitates the iconographic type of Klontza and the Flemish copper engravings.	Master of Panayotis Doxaras

A/A	NAME	Period	Place of Birth	PLACE OF WORK	CHARACTERISTICS	OTHER
19.	Theodoros Poulakis	1620 - 1692	Chania – Crete	Crete – Corfu (died in 1692) – Venice (1644-1657 and 1671-1675)	Follows the models of Klontza and Damaskinos. He is influenced by the Flemish copper engravings of Johannes Sadeler. Baroque, many-figured icons. He holds the tradition in flesh and crackling.	Belongs to the great Cretan painters who traveled abroad
20.	Filotheos Skoufos	16...-1685	Possibly Crete	Crete (Handaka1645) – Corfu (1646/48) – Zakynthos (1665-1685) – Venice (1653)	He used to copy icons made by earlier conservative painters.	Monk. Second-class artist without creative initiatives. Contemporary of Emm. Tzanes and Victor.
21.	Vlastos Ioannis or Mpounialetos	1st half of the 17th c.	Islands of Ionion	Ionion – Venice	Influenced by Em. Tzanes	Priest. Student of Em. Tzanes Mpounialis
22.	Ntzenos Ioannis	Mid 17th c. With beginning of 18th c.	Possibly Crete	Corfu	Influenced by Em. Tzanes and by the Baroque of the time	Member of the cretan family Tze, Tzen or Tzenou (Zen).
23.	Stefanos Tzankarolas	End of 17th c. – beginning of 18th c.	Crete	Crete – Corfu – Venice	Byzantine with Italian elements. Influenced by Emm. Tzanes – manierism – baroque engravings – western models and Italian Renaissance	Priest. His technique is the evolved technique of Em. Tzanes.

A/A	NAME	Period	Place of Birth	PLACE OF WORK	CHARACTERISTICS	OTHER
24.	Konstantinos Kontarinis	1699 - 1732	Crete	Rethymno – Zakynthos – Corfu	Follows Byzantine prototypes and technique of Tzanes. Many of his works have a western character and they do not retain any memory of the Byzantine art.	Contemporary to Emm. Tzanes and Th. Poulakis
25.	Panayiotis Doxaras	1662 - 1729	Kalamata	Ionion – Venice (1690-1715)	Italian influences in technique and composition. Italian Renaissance and great Italian masters. He is the Father of Neohellenic Painting.	Warrior and painter. Student of Leo Mosko (1685-1689). He wrote a theory on painting called "Peri Zoografias" (about painting) (1726)
26.	Nikolaos Doxaras	1705 - 1775	Ionian islands	Venice – Islands of Ionion	Inferior to his father. Having the works of Italian Mannerism and Baroque still fresh in memory, he took one step further in the secularization of religious subjects.	Son of P. Doxaras. He apprenticed in a painting studio in Venice.
27.	Demetrios	18th century	Zakynthos	Zakynthos - Islands of Ionion	Western characteristics	

A/A	NAME	Period	Place of Birth	PLACE OF WORK	CHARACTERISTICS	OTHER
28.	Demetrios Staurakis	18th century	Zakynthos	Zakynthos - Islands of Ionion	Western characteristics	All his work was destroyed at the earthquake of 1953 apart from 3 icons which are displayed in the Museum of Zakynthos and 2 icons in the Church of St Nikolaos of Molos.
29.	Nikolaos Kallergis	18th century	Zakynthos	Zakynthos - Islands of Ionion	Western characteristics	
30.	Nikolaos Koutouzis	1741-1813	Zakynthos	Zakynthos - Islands of Ionion	His work consisted mainly of religious subjects and portraits, while it's being characterized by Italian models and elements of the late Italian Baroque.	Student of Nikolaos Doxaras and possibly of Tiepolo in Venice, for a while.
31.	Nikolaos Kantounis	1767-1834	Zakynthos	Zakynthos - Islands of Ionion	The most important representative of the fourth generation of painters who had definitely turned towards the west. He worked in the style of the late baroque– noticeable first and foremost in the compositions with many figures-compositional ability.	He calls himself an autodidact while in his twenties was ordained also a priest.
32.	Mourdelatos	Late 18th century, early 19th c.	Cephalonia	Cephalonia - Islands of Ionion	Western characteristics	

Appendix II. Bureaucratic procedures (A & B) for sampling permission

A) The decision letter of the Ministry of Culture followed by translation to English

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
ΥΠΟΥΡΓΕΙΟ ΠΟΛΙΤΙΣΜΟΥ
ΓΕΝΙΚΗ ΔΙΕΥΘΥΝΣΗ ΑΡΧΑΙΟΤΗΤΩΝ
ΚΑΙ ΠΟΛΙΤΙΣΤΙΚΗΣ ΚΛΗΡΟΝΟΜΙΑΣ
20ή Εφορεία Βυζαντινών Αρχαιοτήτων

Ταχ. Θυρ.: 189

Ταχ. Δ/νση: Μουσείο Ζακύνθου
Πλ. Σολωμού 3
291 00, Ζάκυνθος
Τηλ.: 2695042714
Fax: 2695029931
E-mail: 20eba@zak.forthnet.gr

Ζάκυνθος, 21-9-06

Αριθ. Πρωτ.: 2148

ΠΡΟΣ: κ. Ελένη Κουλουμπή
Εθνική Πινακοθήκη-
Μουσείο Αλ. Σούτζου
116 01, Αθήνα

ΘΕΜΑ: Χορήγηση άδειας δειγματοληψίας από ζωγραφικά έργα του Μουσείου Ζακύνθου στην κ. Ελένη Κουλουμπή.

ΑΠΟΦΑΣΗ

Έχοντας υπόψη:
1. Τις διατάξεις του Ν. 3028/2002 «Για την Προστασία των Αρχαιοτήτων και εν γένει της Πολιτιστικής Κληρονομιάς»
2. Τις διατάξεις του ΠΔ 191/2003
3. Το Ν. 1622/86
4. Το με αρ. πρωτ. 174/11-9-06 έγγραφο του Τοπικού Συμβουλίου Μνημείων Ιονίων Νήσων
5. Την από 5-7-06 αίτησή σας

ΑΠΟΦΑΣΙΖΟΥΜΕ

Τη χορήγηση άδειας δειγματοληψίας από ζωγραφικά έργα του Μουσείου Ζακύνθου στην κ. Ελένη Κουλουμπή, σύμφωνα με τον πίνακα που υποβλήθηκε στην Εφορεία μας.

Εσωτ. Διανομή: Εργαστήριο Συντήρησης 20ής ΕΒΑ

Η Διευθύντρια της Εφορείας
Ζωή Α. Μυλωνά
Αρχαιολόγος

HELLENIC DEMOCRACY
MINISTRY OF CULTURE
GENERAL ADMINISTRATION OF ANTIQUITIES
AND CULTURAL HERITAGE
20th Ephorate of Byzantine Antiquities

P.O. Box: 189

Address: Zakynthos Museum
Solomou Square 3
291 00, Zakynthos
Tel: 2695042714
Fax: 2695029931
E-mail: 20eba@zak.forthnet.gr

Zakynthos, 21-9-07

Protocol No: 2148

TO: Ms Eleni Kouloumpi
National Gallery-
Alexandros Soutzos
Museum 116 01, Athens

SUBJECT: Permission to Ms Eleni Kouloumpi for sampling procedure from painted objects of Zakynthos Museum

DECISION

Taking into consideration:

1. The orders of the law 3028/2002 "For the Protection of the Antiquities and the Cultural Heritage"
2. The orders of Decree-Law 191/2003
3. The law 1622/86
4. The document of the Local Council of Monuments of the Ionian Islands with protocol no/ 174/11-9-06
5. Your application of the 5-7-06

WE DECIDE

To permit the sampling procedure from painted artefacts of the Zakynthos Museum to Ms Eleni Kouloumpi, according to the table that was submitted to our Ephorate

Internal distribution: conservation Studio of 20th EBA

The Director of the Ephorate

Zoi A. Mylona

Archaeologist

B) The decision letter of the Zakynthos Cathedral followed by English translation

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
ΙΕΡΑ ΜΗΤΡΟΠΟΛΙΣ ΖΑΚΥΝΘΟΥ

Αρ.Πρωτ. 259 Ζάκυνθος 28 Σεπτεμβρίου 2006

Κυρία
Ελένη Κουλουμπή
Αθήνα

Κυρία Κουλουμπή

Δια του παρόντος επιτρέπομε την μελέτη, μέσω της δειγματοληψίας, στον κατάλογο των εικόνων που μας προσκομίσατε, προκειμένου να μελετηθούν οι δυτικές επιδράσεις στην μεταβυζαντινή τέχνη της Κρήτης και των Ιονίων Νήσων.

Οι μελέτη θα πραγματοποιηθεί σε συνεργασία με το Εργαστήριο συντήρησης εικόνων και έργων τέχνης της Ιεράς Μονής Στροφάδων και Αγίου Διονυσίου.

Αναμένομε αντίγραφο της διατριβής Σας, ώστε να γίνουμε και εμείς κοινωνοί των συμπερασμάτων Σας.

Εντολή Σεβ. Μητροπολίτου
Γενικός Αρχιερατικός Επίτροπος

ΙΕΡΑ ΜΗΤΡΟΠΟΛΙΣ ΖΑΚΥΝΘΟΥ

Πρωτοπρ. Παναγιώτης Καποδίστριας

Κοινοποίηση:Εργαστήριο συντήρησης Τηλ. 26950 44126, 6977893785

HELLENIC DEMOCRACY
HOLY CATHEDRAL OF ZAKYNTHOS

Protocol No. 259 Zakynthos 28th of September 2006

Ms
Eleni Kouloumpi
Athens

Ms Kouloumpi

By this letter we allow the study, through sampling procedure, of the icons you applied for, in order to study the Western influences in the post-Byzantine art of Crete and the Ionian islands.

The study will take place in collaboration with the Icons and Artefacts' Conservation Studio of the holy Monastery of Strofadon and St Dionysios.

We expect a copy of your thesis, in order to become aware of your conclusions.

Order of the Reverend Metropolitan Bishop
General Chief Priest Commissioner

P. Panayiotis Kapodistrias

Notification: Conservation Studio Tel: 26950 44126, 6977893785

Appendix III. Health & Safety

All the necessary measures were taken during the experimental procedure. At all times, the author was wearing a laboratory coat, disposable gloves and safety goggles.

All the materials used for the production of the reference samples were natural products, such as oils, vinegar, hen's eggs and pigments. There were no hazards for those materials apart from the lead pigments, which are poisonous. Those pigments were handled with care to avoid contact with skin and to avoid inhalation. When the oven was used for the ageing process of the samples, care was taken to avoid contact with the hot parts of the oven and the samples were handled with heat resistant gloves.

The use of the μRaman, μFT-IR and the SEM/EDX do not have special hazards.

The preparation and treatment of samples for the staining of cross-sections and the gas chromatographic analysis was undertaken with extra care due to the hazardous materials involved. Closed system and ventilation were used at all times. When extremely hazardous solvents, such as pyridine, were employed, extra eye protection in combination with air breathing protection was used.

Appendix IV. Photographs of the icons studied

1. Stavrosis, 14th c., Byzantine Christian Museum

2. Aggelos: St Theodoros The Tiron, 1st half of 15th c., Byzantine Christian Museum

3. Aggelos: St John the Baptist, 1st half of 15th c., Byzantine Christian Museum

4. Aggelos:The entrance of Virgin Mary, 1st half of 15th c., Byzantine Christian Museum

5. Aggelos: Virgin Mary the Kardiotissa, 1st half of 15th c., Byzantine Christian Museum

6.Aggelos: St George, 2nd quarter of 15th c., Byzantine Christian Museum

7. Pavias: The Crucifixion, 2nd half of 16th c., National Gallery

8. Aggelos: Virgin Mary with St Catherine, early 16th c., Monastery of Patmos

9. Virgin Mary holding Jesus Christ, 1540, Monastery of Patmos

10. Damaskinos: Christ the Great Archpriest, 2nd half of 16th c., Byzantine Christian Museum

NO PHOTO AVAILABLE

12. Christ the Great Archpriest, 17th c., Academy of Athens

11. Klontzas (?): The life of Jesus Christ, 2nd half of 17th c.- early 17th c., Byzantine Christian Museum

13. St Nicolaos, Vasileios & Antonios, 17th c., Academy of Athens

14. Tzanes: Virgin Mary Enthroned, 1661, Byzantine Christian Museum

15. Poulakis: Epi soi Chairei, 1660-1690, Monastery of Patmos

16. Poulakis: The draught of Joseph in the pit and his sale to the Israelis, 2nd half of 17thc., Museum of Byzantine Culture

17. Poulakis: The moarning of Jacob for the death of Joseph, 2nd half of 17thc., Museum of Byzantine Culture

18. Poulakis: The sale of Joseph to Pentefris in Egypt, 2nd half of 17thc., Museum of Byzantine Culture

19. Poulakis: Prophet Elias, 2nd half of 17th c., Church of Ano Korakiana, Corfu

20. Poulakis: Archangel Michael, 2nd half of 17th c., Benaki Museum

21. Poulakis: St Spyridon with Scenes of His Life, late 17th c., Benaki Museum

22. The Hypapante, late17th c. – late 18th c., Academy of Athens

23. The Palm carrier, late17th c. – late 18th c., Academy of Athens

24. The Birth of Christ, late 17th c., Academy of Athens

25. Virgin Mary holding Crucified Christ, late 17th c., Academy of Athens

26. The Symbol of Faith, part B', late 17th c., Academy of Athens

27. The Epitaph Lament, late 17th c., Academy of Athens

28. The Crucifixion, late 17th c., Academy of Athens

29. Doxaras (?): Archpriest, late 17th c., Byzantine Christian Museum

NO PHOTO AVAILABLE

30. Kontarinis (?): Allegory of the Holy Communion, 1st half of 18th c., Byzantine Christian Museum

31. Kontarinis: Virgin Mary holding Baby Jesus Christ, 1st half of 18th c., Benaki Museum

32. Tzankarolas: The worshipping of the Shepherds, early 18th c., National Gallery

33. Paradise & Hell, early 18th c., National Gallery

34. Doxaras: Jesus Christi washes the feet of His Disciples, early 18th c., private collection

35. Virgin Mary with Christ, 17th c. (?),Chapel of Virgin Mary "The Lady of the Argil", Kontogenada

36. St John the Baptist, 17th c. (?),Chapel of Virgin Mary "The Lady of the Argil", Kontogenada

37. Jesus Christ - Holy Gate, 17th c. (?),Chapel of Virgin Mary "The Lady of the Argil", Kontogenada

38. Archangel Michael, 17th c. (?),Chapel of Virgin Mary "The Lady of the Argil", Kontogenada

39. Pantokrator, 17th c. (?),Chapel of Virgin Mary "The Lady of the Argil", Kontogenada

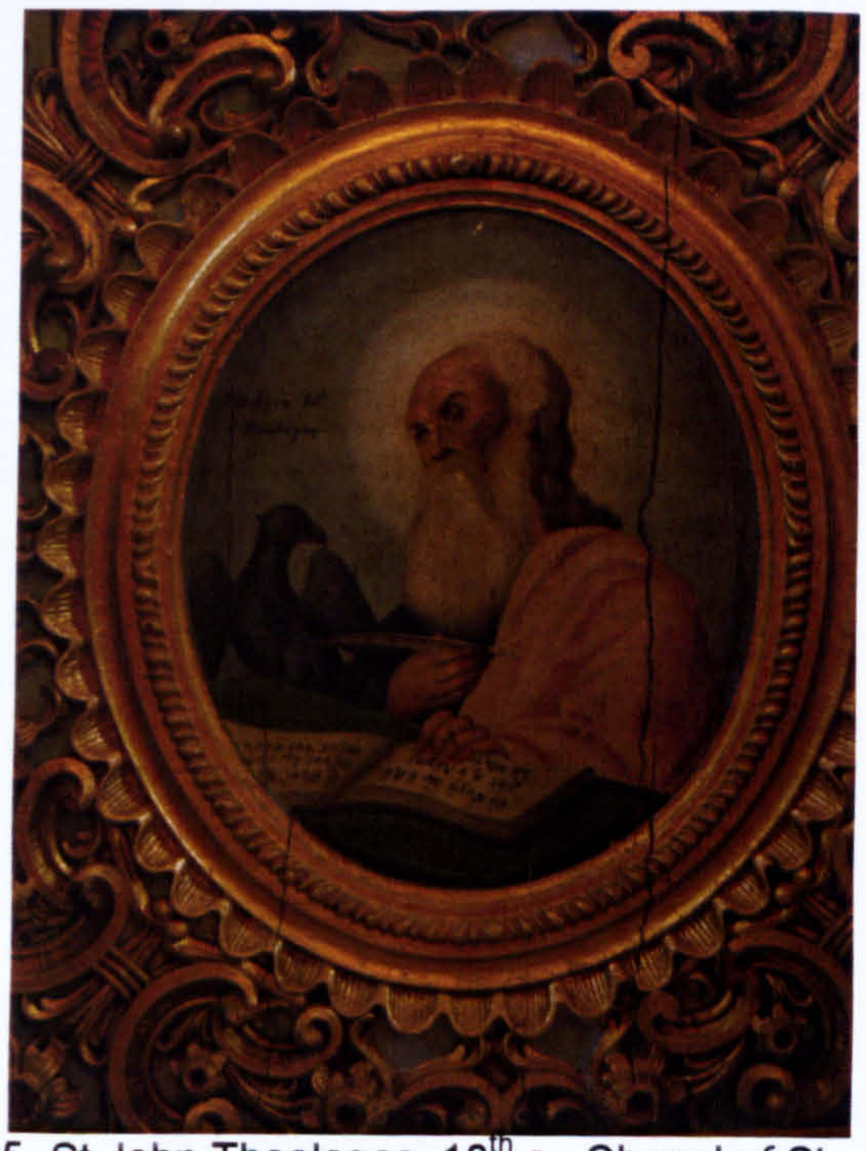
40. Archangel Gabriel, 17th c. (?),Chapel of Virgin Mary "The Lady of the Argil", Kontogenada

41. The Assumption, 17th c. (?),Chapel of Virgin Mary "The Lady of the Argil", Kontogenada

42. The Assumption, 18th c., Chapel of St John Theologos, Kontogenada

43. Archangel Gabriel, 18th c., Chapel of St John Theologos, Kontogenada

44. Jesus Christ, 18th c., Chapel of St John Theologos, Kontogenada



45. St John Theologos, 18th c., Chapel of St John Theologos, Kontogenada

46. St.... (cannot be distinguished), 18th c.(?), Chapel of the Assumption, Oronghi

47. St John the Baptist, St Spyridon, Maria Magdalene & St Gerasimos (?),18th c.(?), Chapel of the Assumption, Oronghi

48. The Assumption, 20th of May 1742, Chapel of the Assumption, Oronghi

49. The Annunciation, mid 18th c., Chapel of the Assumption, Oronghi

50. The Nativity, mid 18th c., Chapel of the Assumption, Oronghi

51. The Palm Carrier, mid 18th c., Chapel of the Assumption, Oronghi

52. The Circumcision of Christ, mid 18th c., Chapel of the Assumption, Oronghi

53. The Baptism of Christ, mid 18th c., Chapel of the Assumption, Oronghi

54. The Raising of Lazarus, mid 18th c., Chapel of the Assumption, Oronghi

55. The Resurrection of Christ, mid 18th c., Chapel of the Assumption, Oronghi

56. The Last Supper, mid 18th c., Chapel of the Assumption, Oronghi

57. The Hypapante, mid 18th c., Chapel of the Assumption, Oronghi

58. The Pentecost, mid 18th c., Chapel of the Assumption, Oronghi

59. The Ascension of Christ, mid 18th c., Chapel of the Assumption, Oronghi

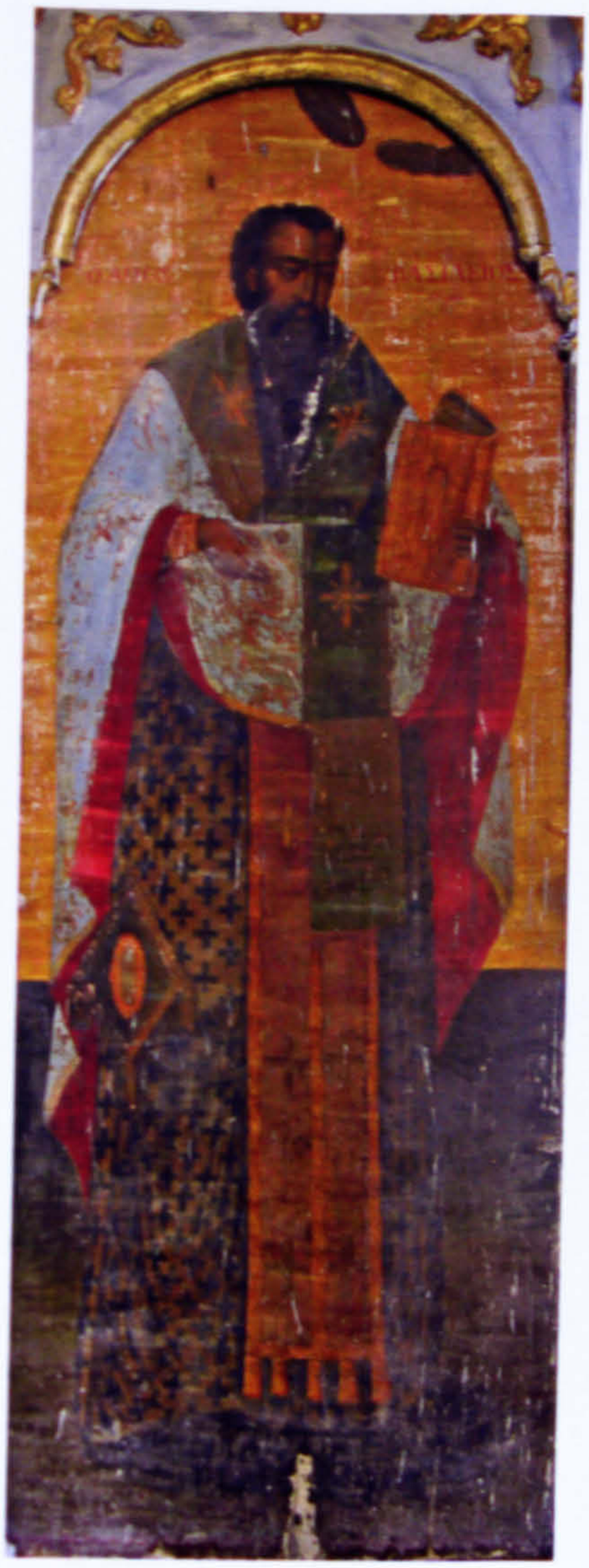
60. The Metamorphosis, mid 18th c., Chapel of the Assumption, Oronghi

61. Mother of God, mid 18th c., Chapel of the Assumption, Oronghi

62. Christ Pantokrator, mid 18th c., Chapel of the Assumption, Oronghi

63. St John the Baptist, mid 18th c., Chapel of the Assumption, Oronghi

64. Diptych of the Prothesis, mid 18th c., Chapel of the Assumption, Oronghi



65. St Vassilios, mid 18th c., Chapel of the Assumption, Oronghi

66. St John Chrysostomos, mid 18th c., Chapel of the Assumption, Oronghi

67. St Gregorios, mid 18th c., Chapel of the Assumption, Oronghi

68. The Crucifixion, mid 18th c., Chapel of the Assumption, Oronghi

69. Mourdelatos: St Gerasimos, 1839, Chapel of St John Theologos, Kontogenada

70. Damaskinos: St John the Baptist, 2nd half of 16th c., Byzantine & Post-Byzantine Museum

71. Gate with St Antonios, 2nd half of 16th c., Byzantine & Post-Byzantine Museum

72. Moskos: The Resurrection, 17th c., Cathedral of Zakynthos

73. Jesus and John, 17th c., Byzantine & Post-Byzantine Museum

74. The Preaching of John, 17th c., Byzantine & Post-Byzantine Museum

75. St Dynati, 17th c., Ecclesiastical Museum

76. Moskos: Archangel, Church of Skopiotissa, Zakynthos

77. Demetrios: The Decollation of St John the Baptist, 2nd half of 17th c., Byzantine & Post-Byzantine Museum

78. Victor: Metamorphosis, 1670, Byzantine & Post-Byzantine Museum

79. Doxaras: Christ the Great Archpriest, 1691, Church of the Lady of the Angels, Zakynthos

80. Doxaras: The Crucifixion, end of 17th c., Ecclesiastical Museum

81. Doxaras: the Adoration of the Shepherds, end of 17th c., Ecclesiastical Museum

82. The Crossing of the Nile, end of 17th c., Byzantine & Post-Byzantine Museum

83. Wooden icon-stand with St Demetrios and Scene from His Life, 18th c., Byzantine & Post-Byzantine Museum

84. St Barbara & Scenes of Her Life, 18th c., Byzantine & Post-Byzantine Museum

85. Archangel Gabriel, 18th c., Byzantine & Post-Byzantine Museum

86. Archangel Michael, 18th c., Byzantine & Post-Byzantine Museum

87. Prophet Ionas, 18th c., Byzantine & Post-Byzantine Museum

88. The Trinity and Archangels, 18th c., Ecclesiastical Museum

89. Kallergis: St John the Baptist, 1st quarter of 18th c., 18th c., Byzantine & Post-Byzantine Museum

90. Kallergis: Christ the Great Archpriest, 1723, 18th c., Byzantine & Post-Byzantine Museum

91. Stentas (?):The Meeting of Mary & Elisabeth, 1723, Church of the Lady of the Angels, Zakynthos

92. Stentas (?): The Decollation of St John the Baptist, 1723, Church of the Lady of the Angels, Zakynthos

93. St Demetrios, 1730, 1723, Byzantine & Post-Byzantine Museum

94. Christ upheld by an angel, 1732, Byzantine & Post-Byzantine Museum

95. Angel, 1732, Byzantine & Post-Byzantine Museum

96. Kallergis: The Entrance of Virgin Mary, 1739, Byzantine & Post-Byzantine Museum

97. Angel with the Symbols of Passion, early 18th c., Byzantine & Post-Byzantine Museum

98. St John the Baptist, 1st half of 18th c., Ecclesiastical Museum

99. St John the Baptist, mid 18th c., Byzantine & Post-Byzantine Museum

100. N. Doxaras or St. Pazigetis: Prophet, mid 18th c., Byzantine & Post-Byzantine Museum

NO PHOTO AVAILABLE
The painting was too long (8m long)

101. Koutouzis: The Litany of St Dionysios, 1766, Ecclesiastical Museum

102. Stavrakis: Christ the Great Archpriest, 1770, Byzantine & Post-Byzantine Museum

103. Koutouzis: Jesus Christ and the Prophets 1, end of 18th c., Ecclesiastical Museum

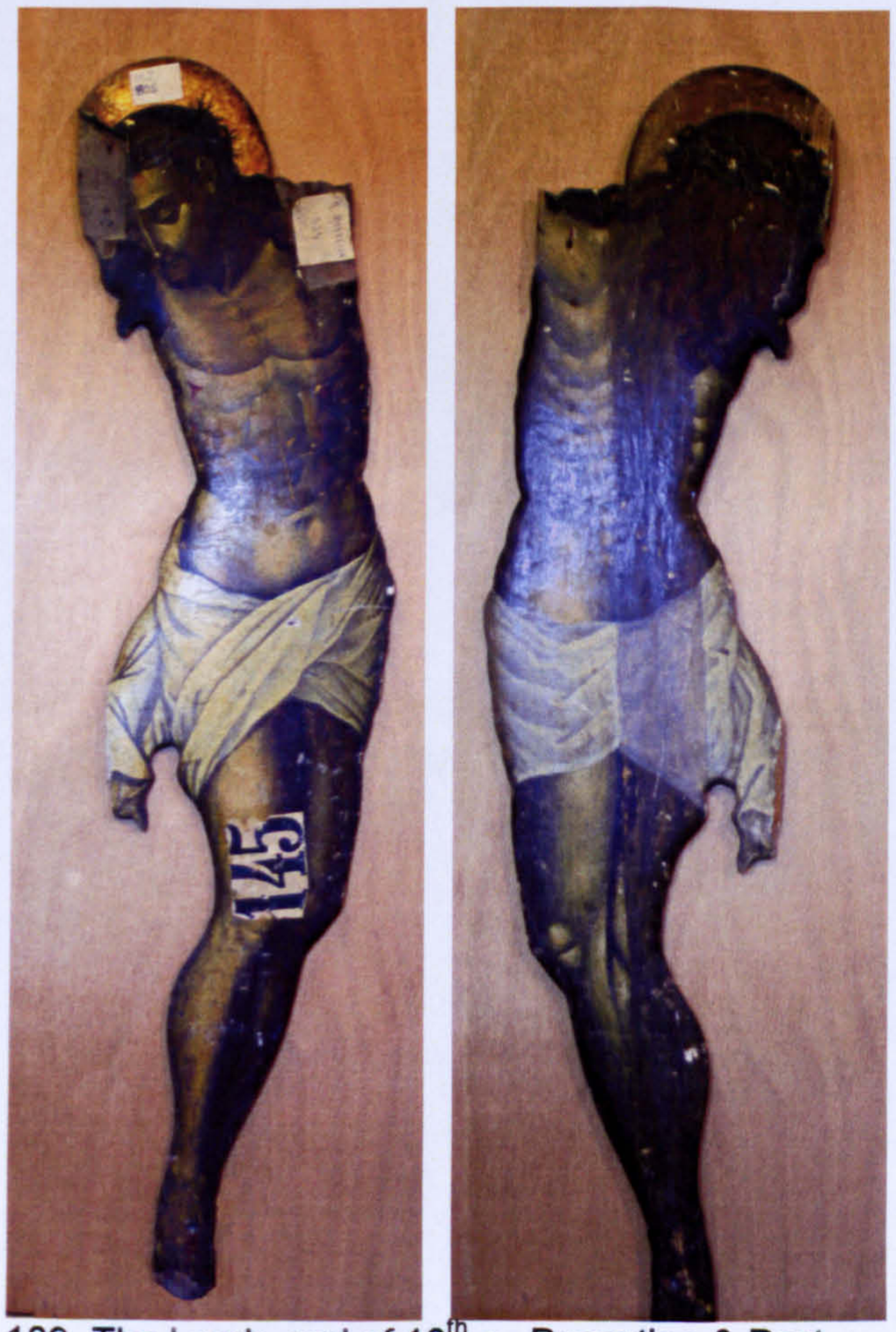
104. Koutouzis: Jesus Christ and the Prophets 2, end of 18th c., Ecclesiastical Museum

105. Koutouzis: The lamb, end of 18th c., Ecclesiastical Museum

106. St Dionysios, end of 18th c., Ecclesiastical Museum

107. Koutouzis: The Dinner at the Emmaous, end of 18th c., Ecclesiastical Museum

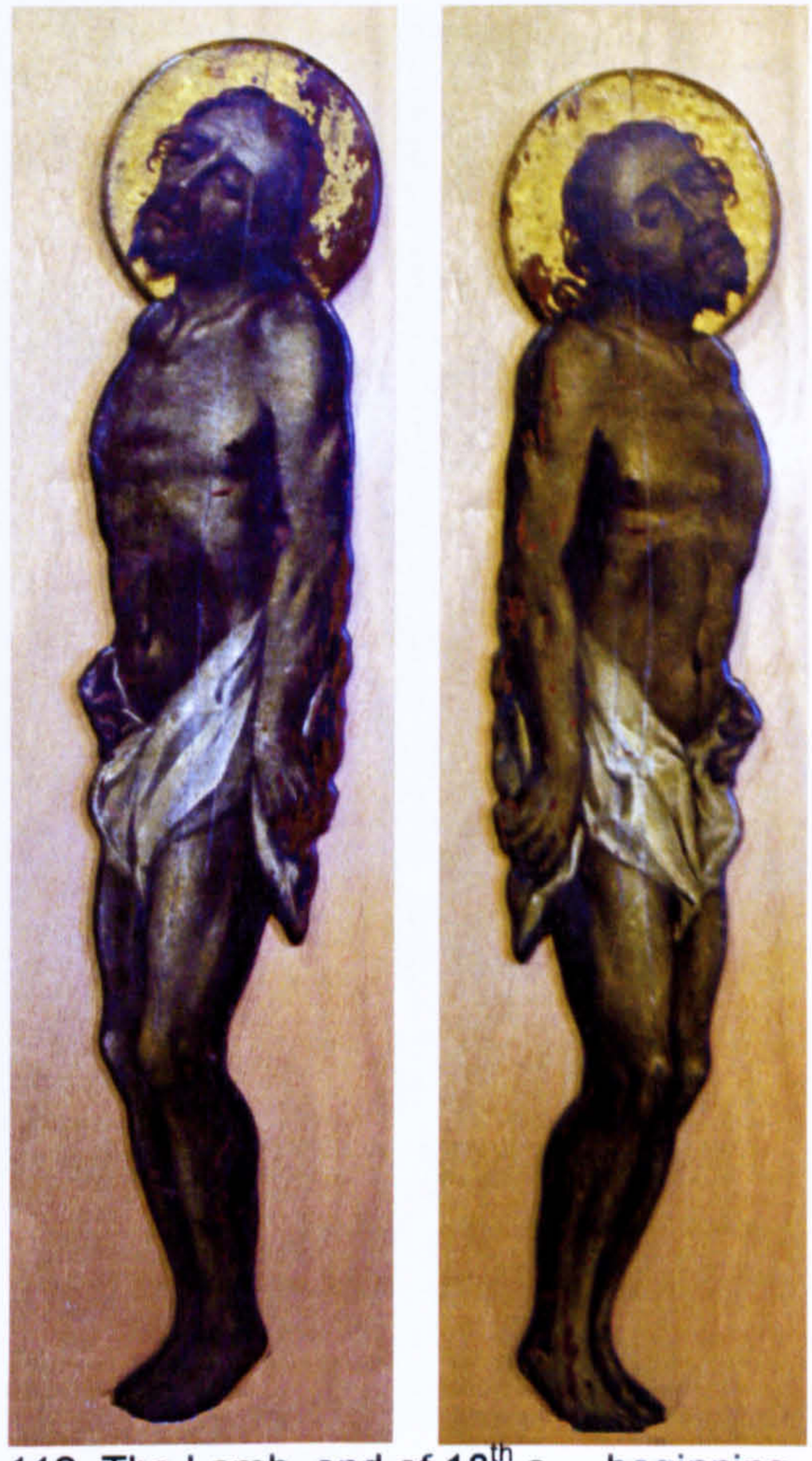
108. Koutouzis: The Annunciation, end of 18th c., Byzantine & Post-Byzantine Museum



109. The Lamb, end of 18th c., Byzantine & Post-Byzantine Museum

110. Ecce Hommo!, end of 18th c. – beginning of 19th c., Byzantine & Post-Byzantine Museum

111. The Annuncuation, end of 18th c. – beginning of 19th c., Byzantine & Post-Byzantine Museum



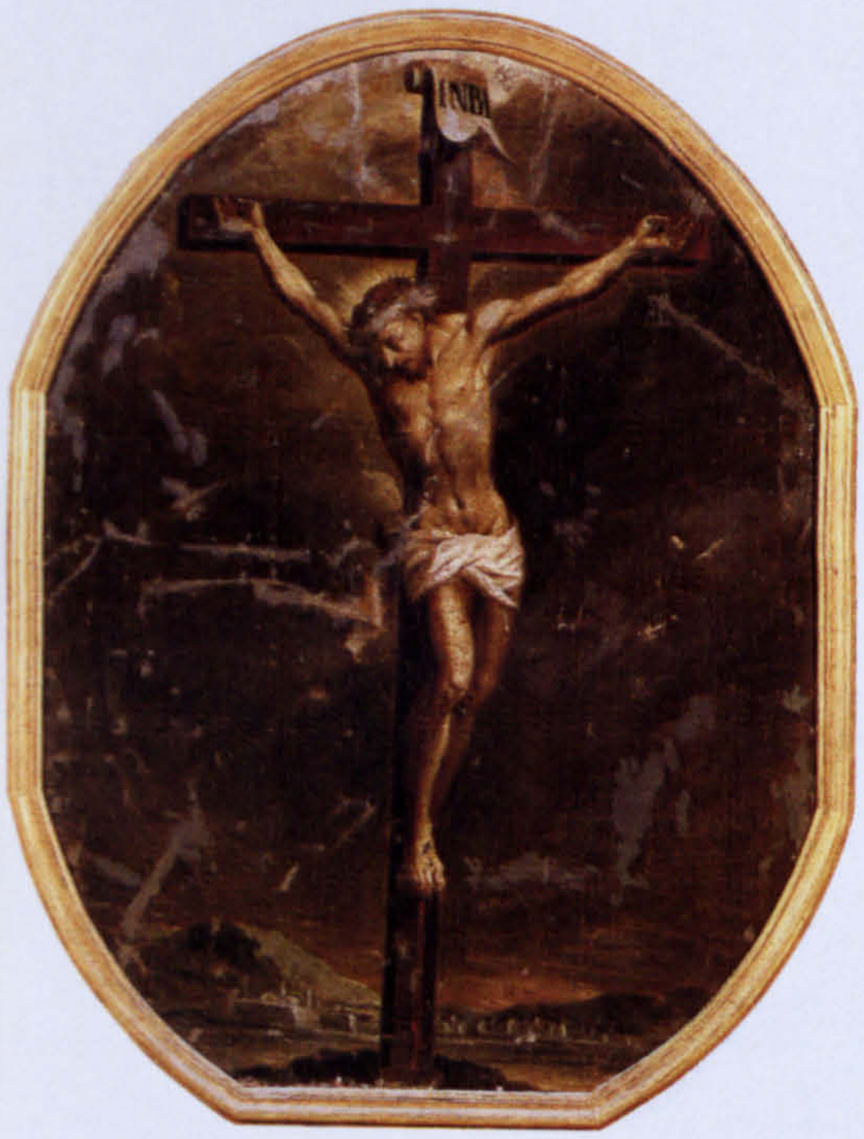
112. The Lamb, end of 18th c. – beginning of 19th c., Byzantine & Post-Byzantine Museum

113. The Worshipping of the Shepherds, end of 18th c. – beginning of 19th c., Ecclesiastical Museum

114. Kantounis: The Baptism, beginning of 19th c., Byzantine & Post-Byzantine Museum

115. Kantounis: The Deposition, beginning of 19th c., Byzantine & Post-Byzantine Museum

116. Kantounis: St Gregorios the Theologian, beginning of 19th c., Byzantine & Post-Byzantine Museum



117. Christ on the Cross, beginning of 19th c., Byzantine & Post-Byzantine Museum

118. Koutouzis: Christ on the Cross on wooden-carved based, beginning of 19th c., Byzantine & Post-Byzantine Museum

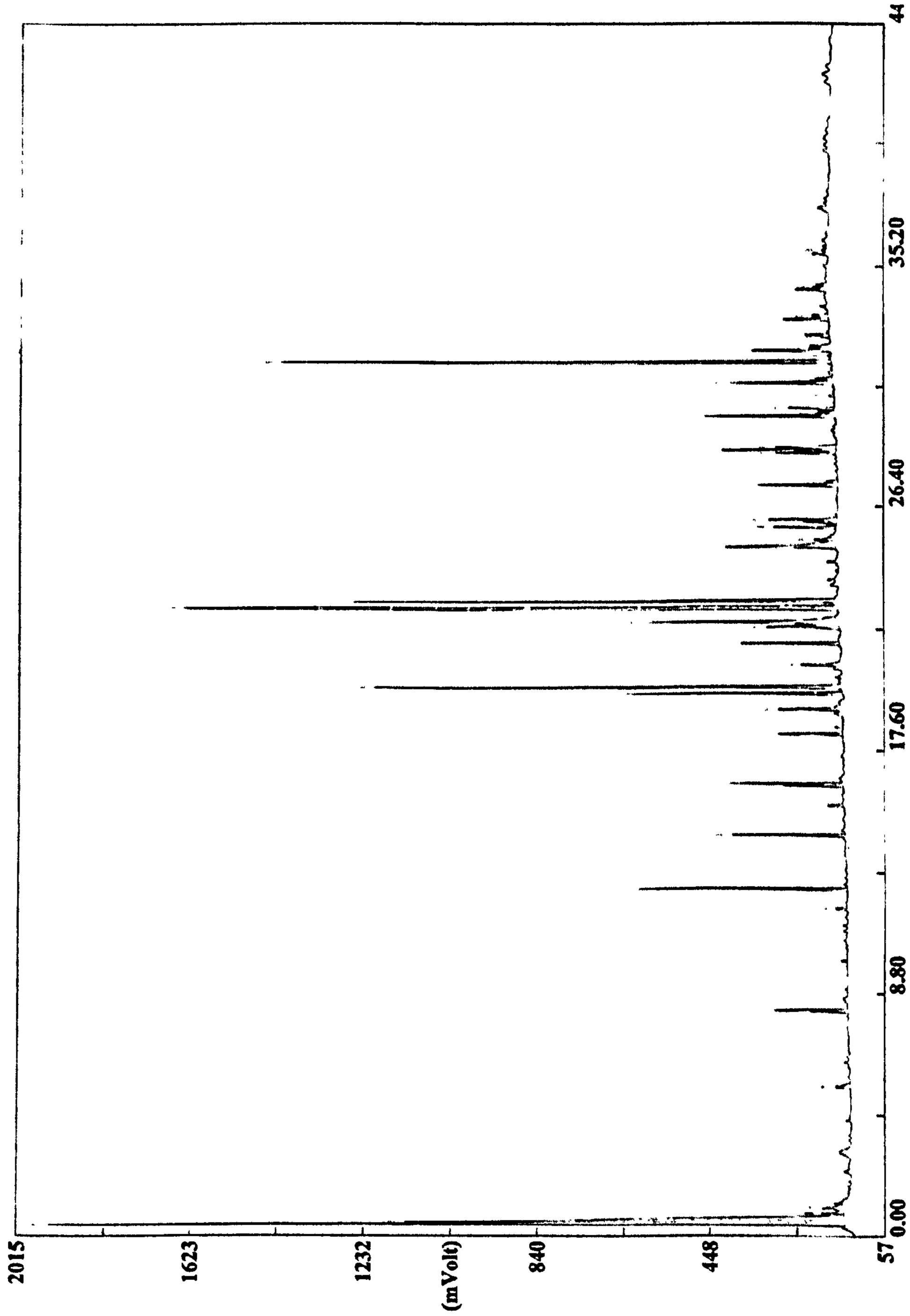
119. The Lamb, early 19th c., Byzantine & Post-Byzantine Museum

120. Kantounis: Evangelist Lucas is painting Virgin Mary

121. Pelekasis: The Annunciation, 19th c.,
Byzantine & Post-Byzantine Museum

Appendix V. Analytical results of reference samples (A & B) with pyrolysis-gas chromatography (Py-GC)

A. Py-GC trace of fatty acid methyl esters



B. Py-GC trace of real sample